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The University of Alberta

Faculty of Graduate Studies and Research

Increased Major Histocompatibility Complex Antigen Expression in Acute Tubular Necrosis of the Mouse Kidney

by

Daniel Arthur Shoskes

A Thesis

Submitted to the Faculty of Graduate Studies and Research

in Partial Fulfillment of the Requirements for the Degree of

Master of Science

In

Experimental Surgery

Department of Surgery

Edmonton, Alberta

Fall 1989

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "Increased Major Histocompatibility Complex Antigen Expression in Acute Tubular Necrosis of the Mouse Kidney",

submitted by Daniel Arthur Shoskes

in partial fulfillment of the requirements for the degree of Master of Science in Experimental Surgery.

Dedicated to the Memory

of my Father

Lew Shoskes

(1920-1983)

Abstract

Acute Tubular Necrosis (ATN) in renal transplant recipients predisposes to rejection and higher rates of graft loss by an unknown mechanism. Induction of Major Histocompatibility Complex (MHC) antigens is reported to precede rejection and increase the T cell response. To study whether ischemic ATN can alter MHC expression, a unilateral model of ATN was produced in the left kidney of male CBA mice by temporary clamping of the vascular pedicle for up to 60 minutes. Class I and II MHC expression was quantified by radioimmunoassay after 1 to 35 days in both kidneys and localized by indirect immunoperoxidase staining (IIP). Specific steady state mRNA for β_2 microglobulin (Class I) and class II were quantified by northern blotting.

By day 1, ATN was evident by histology but there was no consistent change in MHC expression. By day 3, Class I expression was increased in the ischemic kidney by 3 to 6 times over the control both at the antigen and mRNA level. By IIP, the class I increase was localized to tubular epithelial cells. This increase was blocked by either cyclosporine or anti-interferon γ antibody treatment. Starting on day 7 and persisting to day 35, Class II was increased by 1.5 to 3 times for the ischemic kidney over the control, primarily in interstitial cells but also occasionally in tubular cells. This increase was associated with the appearance of

Thy1.2 positive cells in the interstitial areas and was unaffected by cyclosporine.

In mice, unilateral ischemic ATN causes increased MHC expression in tubular cells and the accumulation of an inflammatory infiltrate. This may be one mechanism whereby renal allografts with primary nonfunction become more prone to rejection.

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Abbreviations

α – alpha

ATN - acute tubular necrosis

ATP - adenosine triphosphate

AUC - area under the curve

ß - beta

cpm - counts per minute

DEPC - diethyl pyrocarbonate

DNA - deoxyribonucleic acid

γ – gamma

IIP - indirect immunoperoxidase

i.p. - intraperitoneal

IFN - interferon

IL - interleukin

LPS - lipopolysaccharide

MHC - major histocompatibility complex

mRNA - messenger ribonucleic acid

O.D. - optical density

p.o. - by mouth

PBS - phosphate buffered saline

RIA - radioimmunoassay

rpm - revolutions per minute

RT - room temperature

SDS - sodium dodecyl sulphate

Tx ATN - transplant acute tubular necrosis

Literature Review

Introduction

It is a consistent yet unexplained finding in the renal transplant literature that lack of early renal function predisposes to later graft loss due to rejection. One possible explanation is that the ischemic damage which leads to acute tubular necrosis (ATN) and initial nonfunction also increases the immunogenicity of the organ. The following series of experiments attempts to test this hypothesis in a mouse model of ischemic ATN by examining the changes in magnitude and location of antigen expression in the damaged kidney, compared with the normal contralateral side.

Acute Tubular Necrosis

Acute tubular necrosis is the most common form of acute renal failure[1]. Its many etiologies can be divided into toxic (such as aminoglycoside or organic solvent induced) and ischemic (such as from aortic occlusion or prolonged hypotension). The final common pathology is damage to tubular epithelial cells with tubular obstruction from cellular debris, tubular fluid backleak, reduced glomerular filtration rate, reduced renal blood flow and ultimately clinically evident renal failure[2]. When supportive measures, including dialysis if needed, are instituted early and the etiology removed, ATN is reversible.

ATN in renal tranpslants is almost exclusively due to ischemia. The cadaveric kidney is subject to injury both during storage and reperfusion. The central biochemical derangement during ischemia is accumulation of NADH and depletion of ATP due to the lack of molecular oxygen for oxidative phosphorylation[3]. ATP dependent enzyme systems are rapidly inactivated, leading to an increase in intracellular sodium and osmotic cell swelling. Similarly, calcium accumulates in mitochondria and in the cytosol due to a decrease in Ca^{++}-ATPase activity and decreased Na^{+}-Ca^{++} exchange[4]. Adenosine from ATP is degraded to inosine and hypoxanthine[5] which are important in reperfusion injury. Finally, the dependance of the cells on anaerobic metabolism leads to an accumulation of lactate and hydrogen ions leading to lysosomal and mitochondrial damage.

During reperfusion, when molecular oxygen is again available, the action of xanthine oxidase on hypoxanthine generates the superoxide radical (O_2^-) which can be further reduced to hydrogen peroxide (H_2O_2) and the hydroxyl radical. The superoxide radicals cause irreversible cellular injury by lipid peroxidation of plasma, lysosomal and mitochondrial membranes[6-11].

Renal biopsies of human ischemic ATN typically show focal necrosis of tubular cells. Dilated tubules are often filled with cellular debris (casts). Finally, the development of an interstitial

lymphocytic infiltrate is often seen at the corticomedullary junction and in the vasa recta[12-15].

Transplant ATN and Graft Rejection

In a renal transplant, when function does not begin immediately (in the absence of a technical problem or hyperacute rejection), transplant ATN (Tx ATN) is diagnosed. In cadaveric transplants, the rate of Tx ATN may be as high as 60%. With appropriate supportive measures, most of these grafts eventually begin to function normally.

The deleterious effects of Tx ATN can be divided into early and late. Early problems include the necessity for restarting dialysis, prolonged hospital stay and the performance of more diagnostic tests (renal scan, biopsy) to rule out intercurrent rejection or drug toxicity. The late effects of Tx ATN have been the subject of much controversy over the last 20 years. A consensus is now appearing that kidneys with initial Tx ATN have higher rates of rejection and graft loss than those with immediate function.

In 1967, Williams et al[16] were the first to correlate Tx ATN with graft survival, and they found no effect. Their results were likely influenced by the low graft survival rate (34% at 18 months for cadaveric transplants). Whittaker et al[17] were the first to conclude that initial function significantly improved graft

survival. They believed however that this was due to the morbidity of the investigations and invasive procedures (60% of the ATN group) performed to ascertain the etiology, rather than any specific effects of the ATN. Cho et al[18] reviewed 431 cadaveric transplants and also concluded that Tx ATN impaired graft survival, however they believed that it was the ATN itself to blame. They suggested that ATN could be increasing the immune response or impairing the diagnosis of rejection.

The multicentre studies of Opelz et al[19] have been particularly influential because of the large numbers of transplants (4474) analyzed. They found highly significant correlations between kidney graft function at 1 day, 1 week or 1 month and long term graft survival. They used these findings to conclude that early graft function is a valid indicator of long-term outcome and that therefore new treatment protocols could be assessed after relatively short transplant follow-up. These findings were also confirmed by other centers at that time[20-22]. By contrast, the early Minnesota experience showed no long term effect of Tx ATN on patient or graft survival[23,24]. They postulated that the findings of others that Tx ATN caused rejection may in fact be because severe rejection caused the ATN[24].

The analyses from the Southeastern Organ Procurement Foundation have been very influential because of the large patient numbers from 41 centers, and the use of the powerful

statistical model of multivariate analysis[25-28]. They have consistently found that Tx ATN is associated with decreased graft and patient survival. In 1984, they reported highly significant association between Tx ATN and graft loss from all causes ($p<.00001$), irreversible graft rejection ($p<.001$) and patient death ($p=.012$)[25]. On later follow up, Tx ATN still remained highly significant, having a relative risk of 1.427 for overall graft failure, 1.377 for irreversible graft rejection and 1.267 for patient death[26].

In contrast to the previously noted Minnesota experience, recent reports tend to support the detrimental effect of Tx ATN[29-30]. Heil et al[29] found that Tx ATN correlated with poorer graft survival and attributed this difference between current and previous reports to the overall reduction in graft loss, which had now revealed an effect previously obscured by high overall rejection rates. Canafax et al[30] reported that Tx ATN correlated with poorer graft survival, reducing it from 89% to 72% at one year. Furthermore, cyclosporine was not associated with a higher incidence of Tx ATN, but it did slow the recovery of those kidneys with Tx ATN. These findings were confirmed by other studies in the cyclosporine era[31-33].

There are still a few modern studies which discount Tx ATN as a negative graft survival factor[34,35]. Mendez-Picton et al[34] analyzed the Virginia experience and found that while one year survival for patients with Tx ATN was decreased (52.4% vs

63.0%), it was not statistically significant. The main criticism of this conclusion would be the inclusion of transplants from the 1970's, resulting in very poor overall one year survival rates which could have obscured significant differences. Barry et al[35] reported on 104 cadaveric transplants who received cyclosporine and found no difference in graft survival between Tx ATN kidneys and those that functioned immediately (82.2% vs 82.6%). Patients with Tx ATN did have significantly higher serum creatinines at one month, but there was no difference by 6 months. They attributed their different conclusions to the facts that they used lower doses of cyclosporine and discontinued the drug during OKT3 treatment of rejection episodes.

Recent analyses of transplant data in Ontario and Alberta have supported the deleterious effects of Tx ATN. Halloran et al[36] performed a study of a quadruple therapy protocol in which anti-lymphocyte globulin was used for induction. In this group of 200 consecutive transplant patients, graft and patient survival of 85% and 95% at one year were obtained. Excluding 3 technical failures, grafts with Tx ATN had 75% survival compared with 91% for grafts with good early function. The relative risk for graft loss was 2.86 for Tx ATN. The presence of Tx ATN was the strongest correlate of one year survival, which was also supported by their earlier studies[37-39].

Finally, in a recent review of the University of Alberta renal transplantation database, Madrenas et al (unpublished)

have found Tx ATN to be a significant risk factor for graft failure (relative risk = 1.59, $p<0.027$) and that its effect was additive to that of other significant covariates previously established in their statistical model. This relationship held true regardless of the diagnostic criteria used to define Tx ATN.

In summary, most modern studies of cadaveric renal transplants, including all the multicentre studies with large numbers and all centers using multivariate analysis, find that Tx ATN has a detrimental effect on ultimate graft function and survival. Interestingly, while prolonged warm ischemia and anastomosis times are reliable predictors of Tx ATN, and cold ischemia correlates when very prolonged[40-43], they alone do not correlate with poorer graft survival[30,36]. Anastomosis time has a complex interaction with Tx ATN in predicting graft function, but the full significance of this has not yet been determined.

A mechanism for the increased graft loss in kidneys with Tx ATN has never been proven but there have been hypotheses advanced. One is that not all Tx ATN represents "pure" ATN but rather a combination of ATN with rejection. This could be caused by preformed antibodies not detectable at the time of crossmatch but predisposing to further episodes of rejection. A second possibility is that ischemic damage makes the recovered kidney more sensitive to further insult and vascular shutdown. A third possibility which has been suggested but never investigated[18] is

that ischemia somehow renders a kidney more immunogenic. It is this possibility which prompted these experiments.

Major Histocompatibility Complex Antigens

Introduction

The Major Histocompatibility Complex (MHC) of genes, found in all higher vertebrates, code for molecules that allow thymus derived lymphocytes (T cells) to recognize "self" from "non-self" and coordinate the immune system in its response. Their discovery came about not from organ transplantation research but from experimental oncology. The pioneering work of Gorer[44], Snell[45] and Medawar led to the discovery of the MHC genes, whose products elicited the strongest tissue rejection when incompatible with the host (see Klein[46] and Golub[47] for excellent historical reviews).

Structure

The MHC genes and products are grouped into 3 "classes". Class III genes code for the complement products which act in concert with antigen-antibody complexes to produce cell death and propagate the inflammatory response. Because they are not related to T cell recognition or cellular immunity, they will not be further considered here.

Class I and II products are highly polymorphic transmembrane glycoproteins. Their external portion is divided into domains which have sequence homology with immunoglobulins and the T cell receptor, suggesting a common evolutionary ancestry[48]. The extensive polymorphism, which results in a unique haplotype for each individual in the population, is generated by the existence of multiple alleles for each of the multiple genes.

Class I genes code for antigens (H-2 K,I,S,D in mice; HLA A,B,C in humans) that are expressed on all nucleated cells and platelets, as well as erythrocytes in the mouse[49]. The Class I product is 45,000 daltons with 3 external domains (α1-3), changes in which are responsible for allelic variation. It is bound non-covalently with β_2 microglobulin, an invariant non-MHC encoded peptide of 12,000 daltons[50]. The 3-dimensional structure of human HLA-A2 has recently been determined[51,52]. The region farthest from the membrane (which interacts with T cells) is a platform of 8 ß-strands topped by α-helices. Between these is a groove that provides a binding site for foreign antigens. Most of the genetic polymorphism occurs in the binding site area[52].

Class II genes code for molecules (I-A,E in mice; HLA-D in humans) that are expressed constitutively on a limited number of cell types of the immune system. These include B cells, macrophages, monocytes, activated T cells and some epithelial

cells. The class II molecule is a heterodimer with an α (33,000 daltons) and β (28,000) chain. There is no invariant homologue of $ß_2$ microglobulin. Each chain has 2 globular domain regions. The shorter ß chain contains the allogeneic sites although the α chain does exhibit some structural polymorphism. While class II molecules have not yet been crystallized, current evidence suggests that they bind peptide and interact with the T cell receptor in a manner similar to class I[53].

Function

The function of the MHC antigens is to participate as targets for T cell recognition[54]. T cells will only respond to foreign antigens if they are presented either in conjunction with self MHC (ie. a virally infected cell) or as altered MHC[55] (as in an allograft). T cell subclasses are "restricted" by different MHC classes: $CD4^+$ cells (classically "helper" cells) react with class II and $CD8^+$ cells (classically "cytotoxic/suppressor" cells) react with class I[56,57]. Activated helper T cells proliferate, secrete cytokines (such as interleukin-2 (IL-2) and interferon γ) and coordinate the humoral and cellular immune response. Cytotoxic T cells kill the target cell, a mechanism which may be important in acute graft rejection.

It has been controversial whether T cells recognize antigen and self MHC through two separate receptors or through one[58]. The bulk of experimental evidence pointing to a one

receptor model[59,60] has been lent credence by the aforementioned 3 dimensional structure of Class I[51,52]. If foreign antigen is "seen" by the T cell receptor while held physically in a groove within the MHC molecule, a single receptor to interact with the two molecules seems the most plausible.

The rate of binding of T cells with MHC molecule/antigen complexes is influenced by MHC quantity as well as quality. In normal humans, the amount of MHC of each class expressed varies between organs and cell types[61,62]. It is not surprising that the efficiency of T cell recognition is related to MHC density. In different *in vitro* systems, increased class I expression correlates with more efficient killing by $CD8^+$ cytotoxic T cells[63-66]. Similarly, increased class II expression on the antigen presenting cell leads to more efficient $CD4^+$ cell activation[67-70]. As will be seen, this mechanism plays a role in many physiologic and pathologic responses.

Regulation of MHC Antigens

As previously discussed, different organs and cell types constitutively express varying quantities of MHC antigens. Many factors have been discovered that regulate MHC expression *in vitro* and *in vivo* [71]. These may increase or decrease the amount of each particular class already expressed, may induce the expression of a class not normally expressed constitutively (particularly class II)[72,73] or prevent the induction of expression

by another agent[71]. The primary control of MHC expression is at the level of transcription of mRNA[74] though some posttranscriptional control has been described[75,76].

Induction of MHC products is mediated *in vivo* by lymphokines, the most potent being the interferons (IFN). Type I IFNs (α and β) induce class I but have variable effects on class II[77,78] while type II IFN (γ) consistently induces both classes[79-82]. T cells produce IFN γ when stimulated by mitogens, allo-antigens or IL-2[83] *in vitro*. *In vivo* stimuli of T cell IFN γ release and MHC induction include graft vs host disease [84], sepsis (via lipopolysaccharide (LPS) release)[85,86] and allograft rejection (see below). Other cytokines that induce MHC include tumour necrosis factor[87] (which acts synergistically with IFN γ) and IL-4[88], but whether these are important in MHC regulation *in vivo* is unknown.

Several factors have been shown to decrease MHC expression or at least prevent its induction. The immunosuppressive drugs cyclosporine and the glucocorticoids have negative influences on MHC. Cyclosporine is a cyclic undecapeptide with potent immunosuppressive effects[89]. Among its many effects, it prevents production of the cytokines IL-2 and IFN γ[90]. At the intracellular level, it has recently been discovered that cyclophilin, the protein to which cyclosporine binds, is an enzyme that catalyses the *cis-trans* isomerization of peptidyl-prolyl compounds[91,92], important in protein folding. Its negative

influence on MHC is by inhibition of IFN γ transcription[93,94]. The mechanism of glucocorticoid effects remains unknown, but they do decrease class II expression *in vitro* [95,96]. Other MHC inhibitors include ultraviolet radiation[97] and prostaglandins[98].

MHC Induction and Graft Rejection

Induction of MHC expression by IFN γ appears to be a necessary but not sufficient condition for the rejection of vascularized allografts. Experimental transplantation of rat pancreas[99], heart and kidney[100,101] and of mouse kidney[102] repeatedly shows induction of both class I and II. Rejecting murine kidneys showed class II induction in tubular epithelial cells, which do not express it constitutively[102]. Milton et al studied the kinetics in rejecting rat heart and kidney grafts[100,101] and found the increases to be most prominent between 3 and 7 days post-transplant. Treatment with cyclosporine prevented rejection, abolished the class II increase and reduced the class I increase to control levels, although the inflammatory infiltrate remained unchanged[103]. This effect was presumed to be due to the prevention of IFN γ production by the infiltrating T cells. Conversely, the use of donor specific blood transfusion, an effective immunosuppressive treatment in the rat, prevents rejection while leaving MHC induction and inflammatory infiltration unchanged[104-106].

MHC induction appears to occur in rejecting human allografts as well. In renal transplantation, Häyry et al showed that rejecting grafts had increased class I[107] and II[108] compared with well functioning controls and that this increase made the grafts more immunogenic[108]. This finding has been confirmed by other biopsy series[109-111]. Fuggle et al[112,113] found that class II induction correlated with cellular infiltrate and rejection. All patients with normal class II at day 90 had well functioning grafts 2 years later, whereas 3 of 9 with increased class II had failed. Even 1 year post-transplant, the MHC expressed was of donor type[113]. In addition to kidney, MHC induction has been found to precede rejection in human heart[114] and liver[115] transplantation.

Cellular Activation: Proto-oncogenes and c-myc

Proto-oncogenes are a set of genes that have been preserved through evolution to regulate mitosis and differentiation. Amplification, point mutation or translocation of these genes contribute to malignant transformation of the host cell[116,117]. Thus cellular proto-oncogenes are converted to cellular oncogenes (c-onc) which may be carried by viruses (v-onc).

c-myc is a proto-oncogene found in all vertebrates that is homologous to the oncogene of the avian myelocytomatosis virus MC29[118]. It encodes for a nuclear protein that helps

regulate DNA synthesis[119-121]. *In vitro,* the stimulation of tumour cells[122] or T lymphocytes[123-124] results in increased c-myc transcription. *In vivo* , c-myc expression has been found to be increased in kidneys in autosomal recessive polycystic disease[125], as well as in ischemically damaged liver[126].

The expression of c-myc has not been studied in ischemic ATN in any published animal models. It was chosen for use with this model to allow for a molecular measurement of cell activation during the recovery phase that was independent of MHC expression.

Animal Models of Ischemic ATN

Most research into ischemic ATN has used as subjects either rabbits or rats. The rabbit is large, easy to operate on, and develops pathologic changes similar to humans[127,128]. Similarly, temporary unilateral ischemia in the rat provides a reversible period of ATN beginning 1-3 hours after clamp removal and lasting from 4-8 weeks[129-132]. Ferwana et al[133] found that the most reliable ischemia is produced when the entire renal pedicle rather than just the renal artery is occluded.

Unilateral renal ischemia in the mouse has been used very rarely as a model of ATN. While they reproducibly develop the disease, the necessity for magnification to adequately see the renal vessels makes it more cumbersome to do. The ATN

produced is similar to that in humans with the exception that progressive renal fibrosis occurs 3 months after recovery of the ischemic kidney[134]. The advantages of using the mouse include availability of well characterized inbred strains, cost, and availability of monoclonal antibodies/molecular probes for the study of MHC expression.

Materials and Methods

Surgery

Mouse strains used were either male CBA or female nudes (BALB/c background) aged 4-15 weeks. Animals were anesthetized with Avertin (2,2,2-tribromoethanol in tert-amyl alcohol) i.p. The left renal pedicle was exposed via a midline incision and occluded with a clamp for between 15 and 60 minutes (plate 1). During clamping, the viscera were kept moist with warmed salined. Visual confirmation of reperfusion to the left kidney and lack of inadvertant damage to the right was made prior to closing the incision in 2 layers with 5-0 Dexon.

Animals were sacrificed with ether and cervical dislocation at selected time points after surgery ranging from 5 minutes to 35 days. Each kidney was divided into 4 samples which were individually labelled and processed for radioimmunoassay, immunohistochemistry, mRNA extraction or light histology.

Light Histology

Samples were fixed in 10% formalin and embedded in paraffin blocks for sectioning. Slides were stained with hematoxylin and conter-stained with eosin.

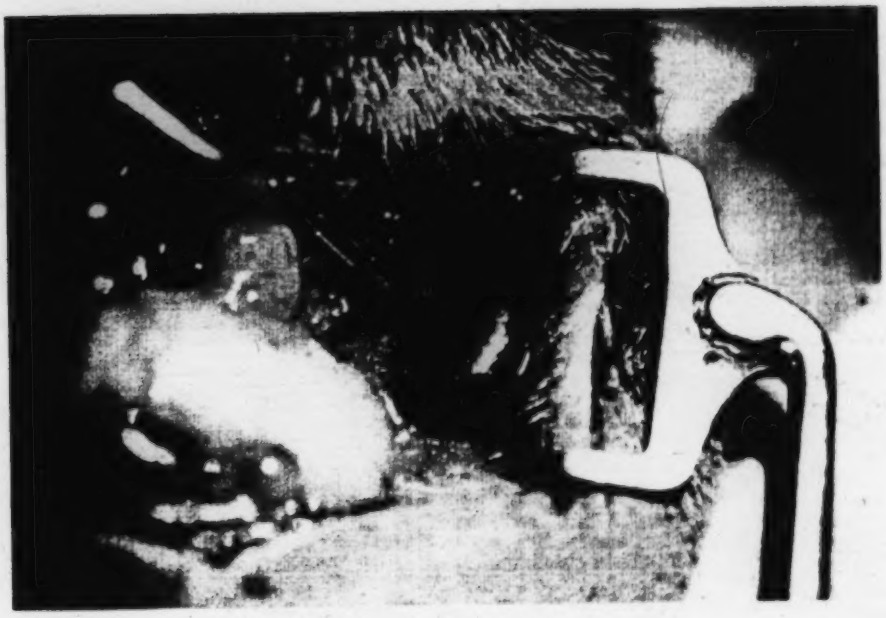

Plate 1: ATN Surgery. Note clip on left renal pedicle

Antibodies

All monoclonal antibodies were grown in our laboratory as ascites tumours or as supernatants from hybridomas supplied by American Type Culture Collection (Rockville, MD). They were purified over a protein A column and adjusted to a protein concentration of 1 mg/ml. The details of the antibodies are provided in table 1. The goat anti rat and goat anti mouse peroxidases were supplied by Cooper Biomedical Scientific Division (West Chester, PA) and were polyclonal $F(ab')_2$ fragments of IgG heavy and light chains.

Radioimmunoassay (RIA)

Samples were snap frozen in liquid nitrogen and stored at -70C. Each experiment contained a control kidney of a different MHC genotype from the experimental group. Each sample was placed in a pre-weighed tube containing 1 ml of Phosphate Buffered Saline (PBS) and homogenized. Each tube was then filled with PBS and centrifuged at 15000 rpm for 20 minutes. The supernatants were aspirated, the tubes weighed, and the pellet weight determined by subtracting the original test tube weight. One ml of PBS was added to each tube and the pellet resuspended by homogenation. A further volume of PBS was added to bring the tissue concentration to 10 mg/ml. A volume of 0.5 ml (5 mg) was then added to small test tubes in triplicate for each antigen to

Hybridoma	Specificity	Antibody Class	Reference
11-5.2.1.9	I-A^k	Mouse IgG_{2b}	135
11-4.1	H-$2K^k$	Mouse IgG_{2a}	135
30-H12	Thy1.2	Mouse IgG_{2a}	136
R4-6A2	Murine IFN-γ	Rat IgG_1	137
MK-D6	I-A^d	Mouse IgG_{2a}	138
34-4-20S	H-$2D^d$	Mouse IgG_{2a}	139

Table 1: Details of Monoclonal Antibodies Used

be tested. These tubes were centrifuged at 3000 rpm for 20 minutes and the supernatants discarded.

Monoclonal antibodies purified and radiolabelled with ^{125}I were prepared in our laboratory. For CBA mice, anti-H-2K^k (Class I) and anti-I-A^k (Class II) were used. These were diluted in PBS with 10% normal mouse serum so that a 100 µl sample would give between 100,000-125,000 counts per minute (cpm) on the gamma counter. One hundred µl of the appropriate antibody was added to each tube containing the 5 mg pellet of tissue and incubated on a rocker on ice for 1 hour. One ml of PBS was then added to each tube to stop the reaction. The tubes were centrifuged at 3000 rpm for 20 minutes, the supernatants discarded and the remaining tube and pellet placed in the gamma counter. Counts of the control tissue were automatically subtracted from experimental data before calculating a mean of the triplicated samples.

Immunoperoxidase Staining

Samples were snap frozen in Tissue Tek™ and frozen sections cut to 5 µm thickness at -20C. Slides were fixed in acetone for 10' at 4C then washed twice in PBS. They were then incubated with a 1:20 dilution of normal goat serum for 20' at room temperature (RT) followed by two further washings. Next they were incubated with the appropriate monoclonal antibody (Class I, II, Thy1.2) at a 1:20 dilution for 60' at RT. One control

slide received PBS instead of antibody. After 3 washes, they were incubated with the appropriate peroxidase (Goat anti mouse for MHC, goat anti rat for Thy 1.2) at a 1:25 dilution for 30' at RT. After the final 3 PBS washes, the peroxidase was developed with a solution of diaminobenzadine (5 mg in 10 ml 0.05 Tris-HCl with 50 µl of 3% hydrogen peroxide) for 10' at RT. Slides were then washed in distilled water, stained with hematoxylin, washed in alcohol and xylene and finally mounted with Permount.

mRNA Extraction

Tissues were snap frozen and stored at -70C. Samples were then pooled for each group and homogenized in a solution of 4M guanidinium HCl with 8µl ß-mercaptoethanol per ml. The tubes were centrifuged at 10,000 rpm for 10' and the supernatants layered on 3.5 ml of Cesium Chloride (5.7M). These tubes were then centrifuged at 24000 rpm for 15 hours. The RNA pellets were flushed with Diethyl Pyrocarbonate (DEPC) water and added to 150 µl of 0.1% Sodium Dodecyl Sulphate (SDS). A chloroform extraction was performed and the aqueous layer added to a fresh tube. Samples were then precipitated with 15 µl of 3M sodium acetate and 2x volume ethanol. Following centrifugation, the pellet was dried in a vacuum and then reconstituted with 100 µl 0.1 SDS. Optical density readings were then taken at a wavelength of 260 nm to determine the concentration of RNA in each sample.

Northern Blot

A gel was prepared by mixing 30 ml of a boiling 1.5% solution of agarose with 5.4 ml formaldehyde and 3 ml RNA buffer. It was poured when cool and wells created with a comb. It was then prerun at 40V.

RNA samples were prepared by adding 20 µg of RNA (by O.D. reading) to a solution of 1.0 µl gel buffer, 3.5 µl formaldehyde, and 10µl of formamide. These were heated at 60C for 10' then rapidly cooled in ice. Two µl of ethidium bromide with bromophenol blue was added to each solution and the total volume added to its appropriate well in the gel. The gel was run at 10-20V overnight.

The gel was then photographed under ultraviolet light to ensure adequate electrophoresis. It was then placed on a northern transfer apparatus using Hybond™ nitrocellulose paper for the transfer and 10x Sodium Chloride/Sodium Citrate (SSC) as the buffer medium. The following day, the blot was washed in 6x SSC then baked at 80C for 2 hours.

Dried blots were placed in a plastic pocket and incubated in a pre-hybridization solution for 3 hours at 42C. The appropriate cDNA probe, radiolabelled with ^{32}P was added and incubated at 42C overnight. The blot was then washed in decreasing concentrations of SSC buffer with 0.1% SDS until geiger counter

readings were appropriate in the area of study. An autoradiograph was then made by placing the blot against undeveloped film and storing at -70C until ready for developing.

Class I was assayed with a β_2 microglobulin cDNA probe[140] and Class II with an A-α c-DNA[141]. c-myc proto-oncogene was assayed with a murine c-myc probe[142]. Actin was used to ensure that an equal quantity of tissue was used in each sample[143].

Drugs

1) Cyclosporine - supplied by Sandoz (Basel, Switzerland) in an olive oil base. An appropriate dilution was made in olive oil so that 0.1 ml would give a dose of 100 mg/kg. This was given p.o. to the animals starting on the day before surgery (or other treatment) and continuing until the last day. When animals displayed signs of neurotoxicity, the day's dose would be omitted for all animals.

2) Lipopolysaccharide (LPS) - Salmonella derived, a solution of 250 µg/ml was made by adding 2 mg to 8 ml sterile saline and inactivating it at 60C for 5 hours. Animals were given 0.1 ml (25µg) i.p.

3) Anti-interferon gamma antibody - a monoclonal neutralizing IgG_1 from mouse hybridoma cell line R4-6A2[137]. Injected i.p. 0.1 ml daily.

Statistics

Comparison of RIA results within animals was done by a paired t test. Comparisons between groups was done by an unpaired t test. For the comparison of data between different experiments, the results were expressed as a percentage of the control tissue for each run. These data were compared using the non-parametric Wilcoxon rank sum test.

Results

Surgery Produces Unilateral Histologic ATN

The first experiments tested different vascular clamping times to assess their efficacy in producing ATN. It was found that 15 to 30' of ischemia failed to produce consistent changes, 45' produced changes in approximately 75% of the affected kidneys and 60' produced consistent and severe ischemic changes in about 90%. Sixty minutes was then used for subsequent experiments.

Gross examination of the kidney at time of animal sacrifice showed no abnormalities in any of the control (right) kidneys or those undergoing sham surgery. The ischemic (left) kidney appeared grossly abnormal after 24 hours with maximal changes after 3 days. These changes included swelling, cortical pallor, medullary vascular congestion and inflammatory plaques on the vascular pedicle (Plate 2). By 5 days only 50% of ischemic kidneys could be distinguished grossly while by day 7 and later the left kidneys appeared grossly indistinguishable from the right.

Light microscopy revealed that in all instances, the right kidneys appeared normal. As well, both kidneys in sham operated controls were undamaged. Five minutes after surgery, the left kidneys appeared normal. By 6 hours, there was isolated thinning of tubular epithelial cells but no evidence of tubular obstruction (plate 3). Beginning at 18 hours and peaking at 3

Plate 2a

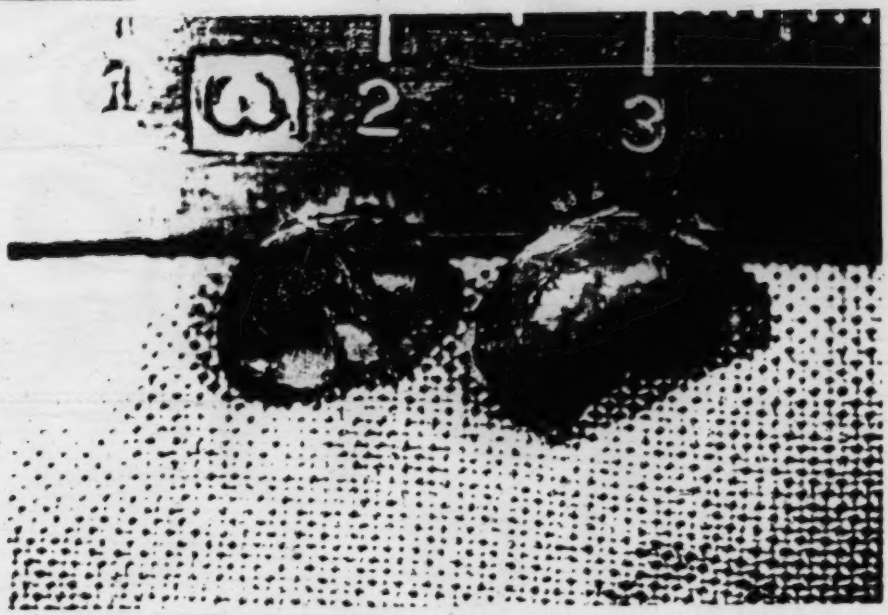

Plate 2b (Cross Section)

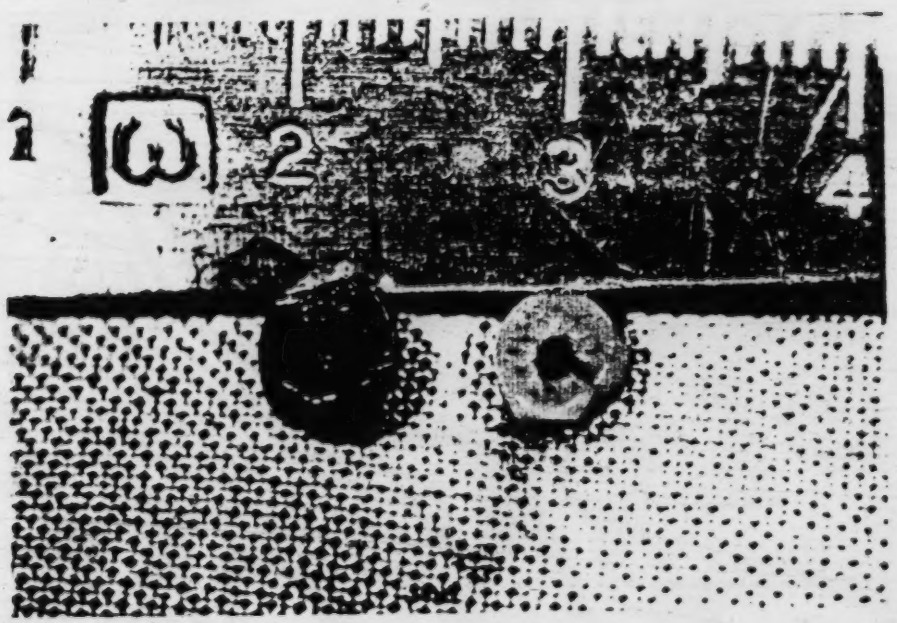

Plate 2: Gross appearance of kidneys (ischemic kidney on right)

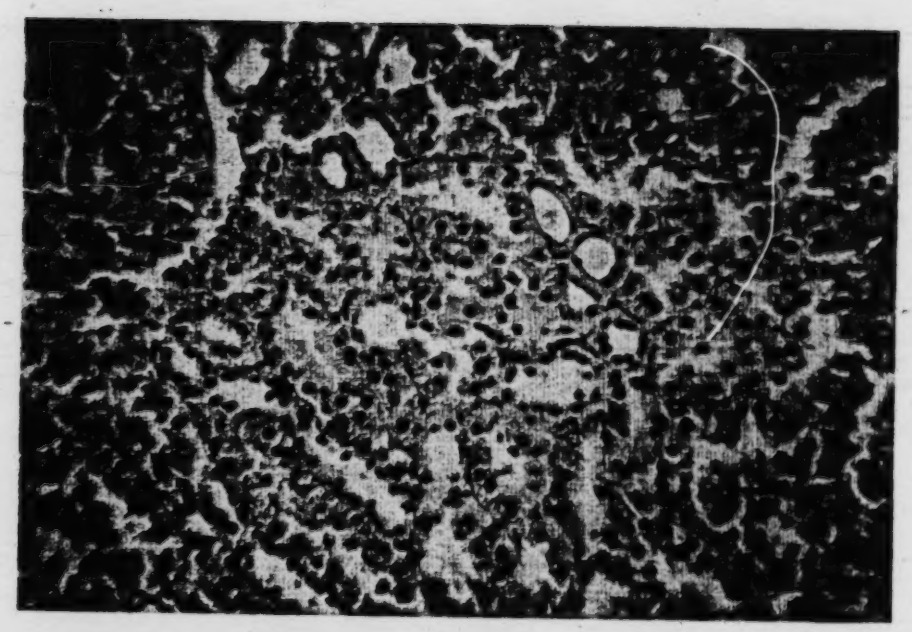

Plate 3: Photomicrograph of ischemic kidney 6 hours after clamp removal. Note early dilatation of tubules (H and E stain 25x)

days, there was widespread tubular dilatation, epithelial cell necrosis and tubular obstruction (plate 4a) with pink staining debris ("casts") (plate 4b). By day 7, tubular dilatation was still present but most lumina were cleared of casts. At this time, hyperplasia of interstitial cells was noted (plate 5a). By days 14-35, the tubules returned to a normal appearance, but the interstitial hypercellularity persisted with the production of frank lymphocytic infiltrates in many specimens, most pronounced on day 21 (plate 5b).

Electron microscopy confirmed the typical findings of ATN. Again, the control kidneys all appeared normal. As seen in plate 6, there was tubular dilatation with loss of the brush border and a minor degree of simplification of the basolateral membrane. Examination of the glomeruli revealed some fusion of the foot processes bilaterally, but this is a normal finding in the mouse kidney.

Serum creatinine and blood urea levels done at the time of sacrifice in selected animals from control and experimental groups showed no elevation, even in the most severe cases of ATN by histology (data not shown).

Control Surgery: MHC Unchanged Between Sides

As seen in figure 1, there was no change in MHC expression by RIA following sham surgery, as quantified by the

Plate 4a (10x)

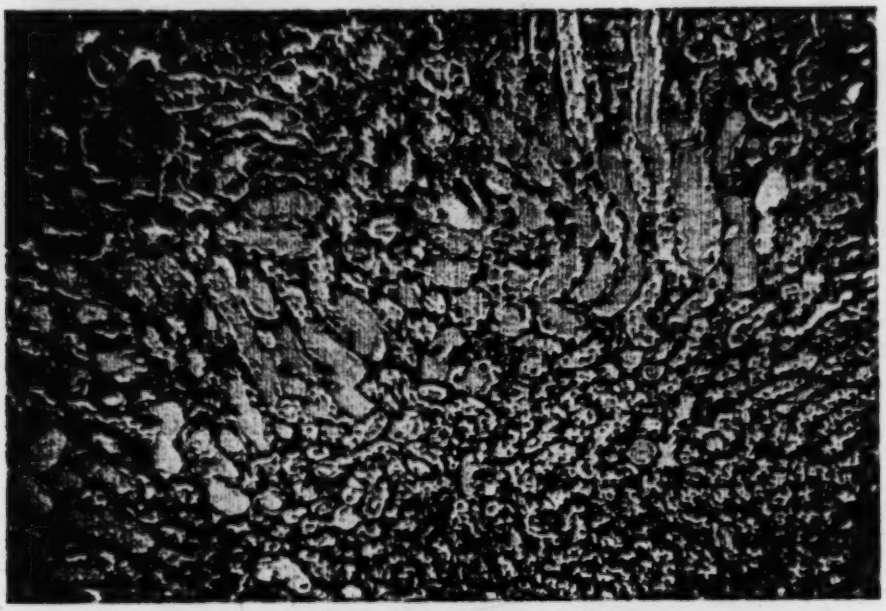

Plate 4b (40x)

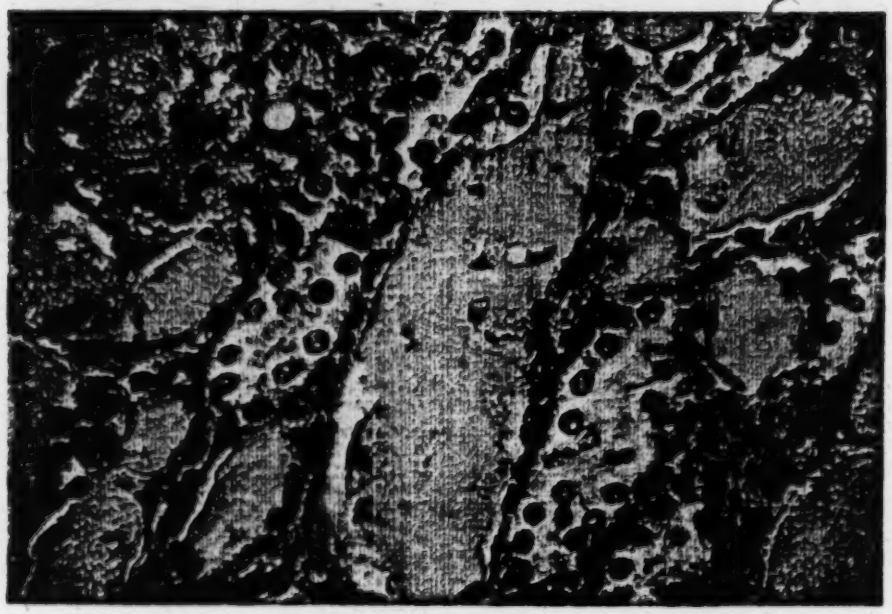

Plate 4: Photomicrographs of ischemic kidney at day 3

Note tubular obstruction and dilatation (H and E stain)

Plate 5a Day 7 (25x)

Plate 5b Day 21 (25x)

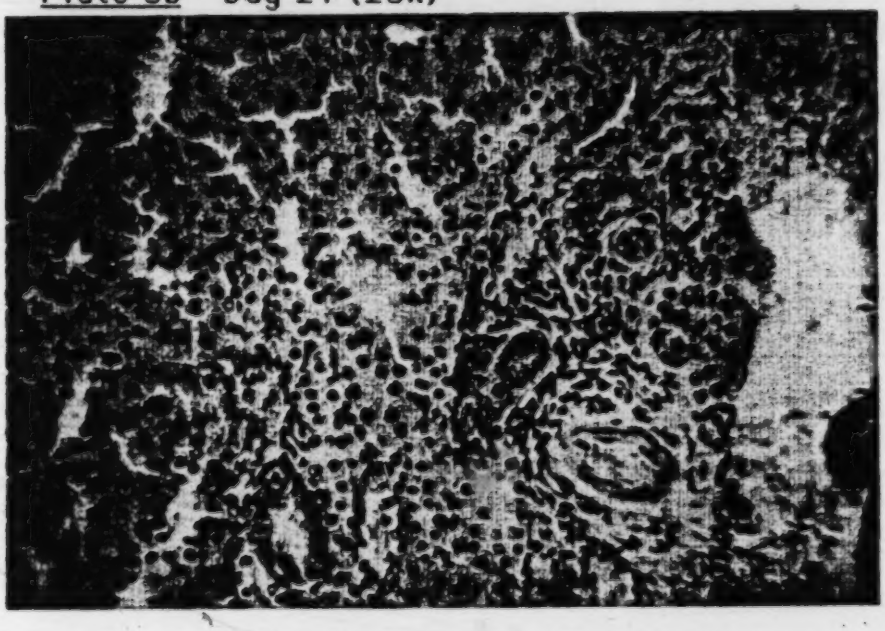

Plate 5: Photomicrographs of ischemic kidney on days 7 and 21. Note progression of interstitial infiltrate and normal appearance of tubules (H and E stain)

Plate 6a: Control (right) Kidney (2100x)

Plate 6b Ischemic (left) Kidney (1500x)

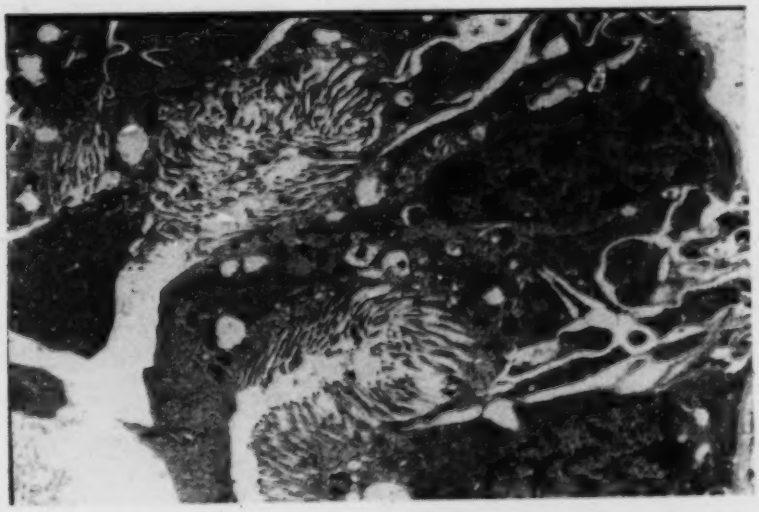

Plate 6: Electron Microscopy of Ischemic and Control Kidneys on Day 3.

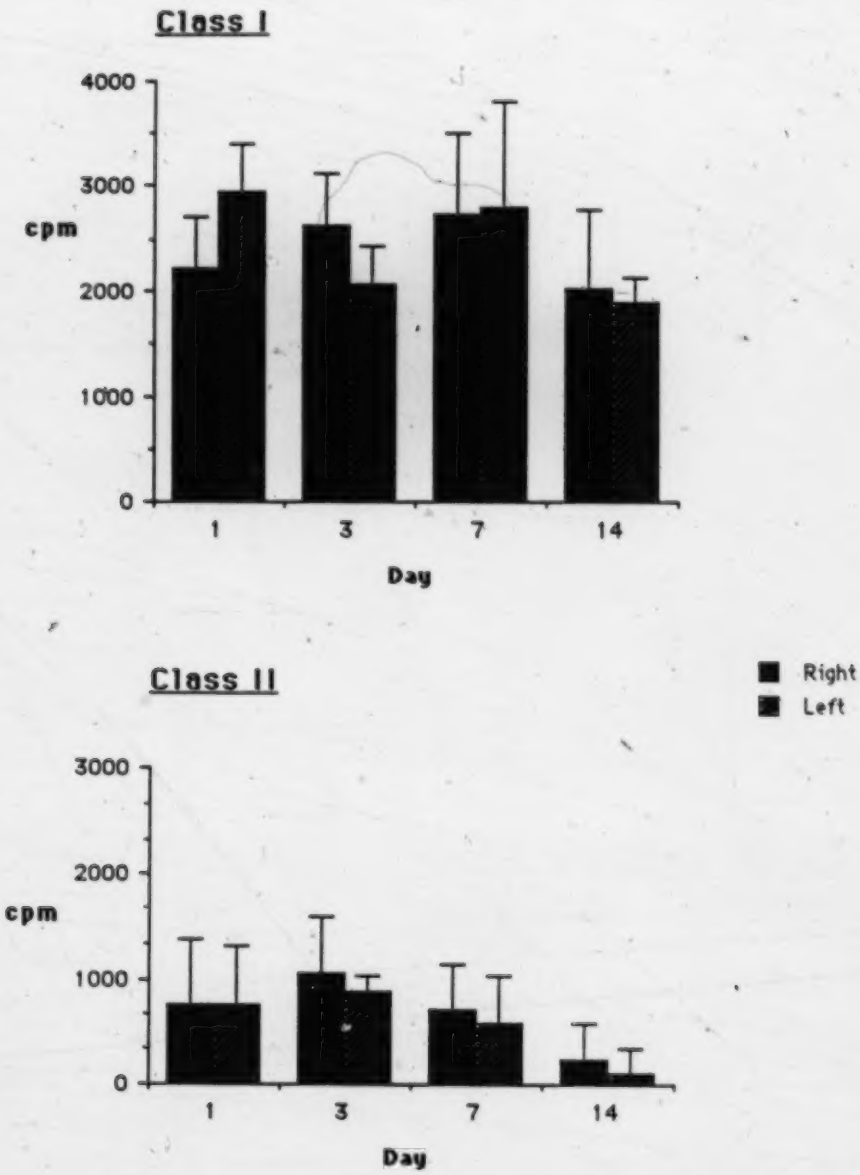

Figure 1: MHC Expression in Sham Surgery

cpm's of specific radiolabelled monoclonal antibody bound to the tissue. There was inter-animal variation accounting for the large standard errors, but no left/right variation within individual animals. Immunoperoxidase staining showed low level class I tubular expression, equal on both sides, and no class II staining. As well, 15' of clamping the left renal pedicle resulted in no histologic changes nor MHC induction.

MHC Changes With 45' Clamping

The results of 45' clamping over 1 to 3 days are presented in figures 2 and 3. At 1 day, there was no change in MHC expression between the sides. By day 3 however, class I was increased by almost 2x in the ischemic (left) kidney over the control side. Class II was unchanged from days 1 to 3. The breakdown of class I for individual animals on day 3 (figure 3) showed that some were increased 2-3x (A, B, C) and one was increased only slightly (D). Comparison with the individual histologic specimens revealed that the animal with no increase also had a lesser degree of ATN. This prompted the switch to 60' ischemia in subsequent experiments.

Tubular Induction of Class I on Day 3

Experiments looking at days 0 to 3 with 60' clamping confirmed that class I expression was increased in the ischemic kidney and class II unchanged. A typical experiment is shown in

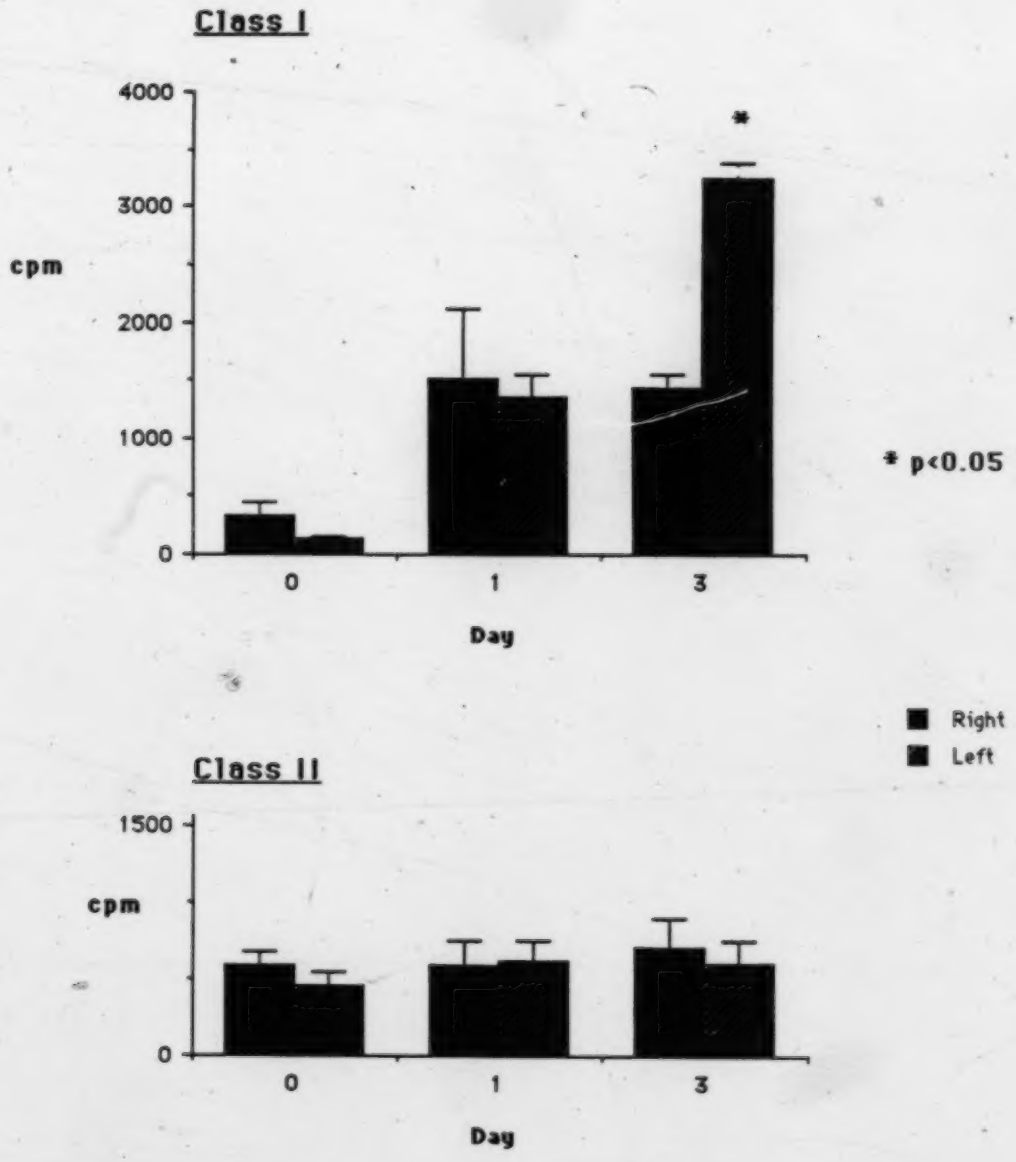

Figure 2: MHC Expression with 45' Clamping

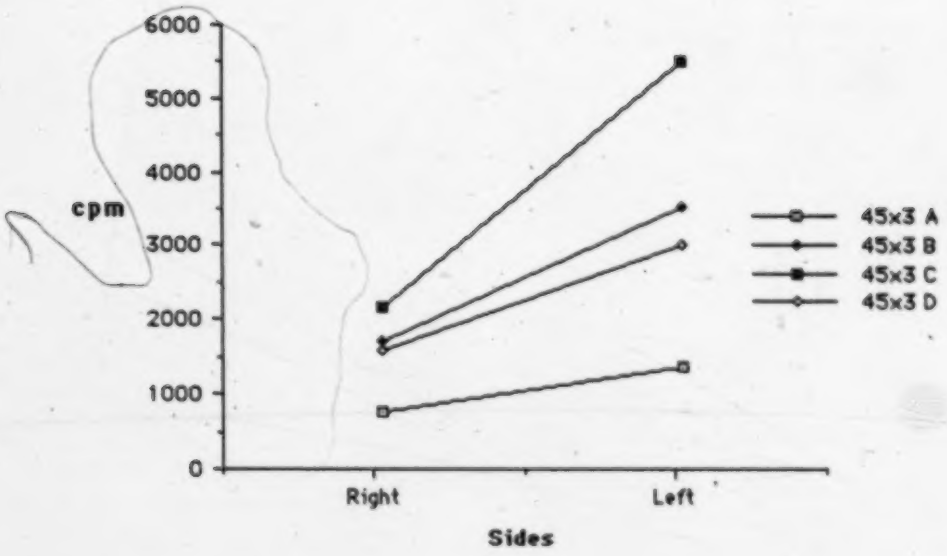

Figure 3: Class I on Day 3 with 45° Clamping

figure 4. Note that the ischemic kidney had increased class I expression over the control side, but not as high as the positive control (LPS), which increased MHC systemically. As seen in figure 5, 60' clamping produced increased class I in all the animals, although the degree of induction still varied with the severity of histologic ATN.

The results of immunoperoxidase staining for class I are shown in plate 7. The increased class I expression was localized to the tubular epithelium. Unlike the uniform staining seen with a systemic agent such as LPS, the increase in ATN was patchy. The limitations of frozen sections on histologic detail made it impossible to distinguish whether the stained tubules were those that were damaged and dilated or those that had remained intact. Neither class II nor Thy1.2 staining was increased in the day 3 ischemic kidney (data not shown).

Gene Activation in Early Ischemic ATN

A northern blot probed with β_2 microglobulin (class I) and c-myc is reproduced in plate 8. The upper row shows that c-myc expression is first detected at 6 hours in the left kidney, is present in both kidneys at 12 hours (L>R) and peaks in the left kidney at 24 hours. By 72 hours, the left is still increased over the right but both are less than at 24 hours. Figure 6 shows this data quantified by densitometry.

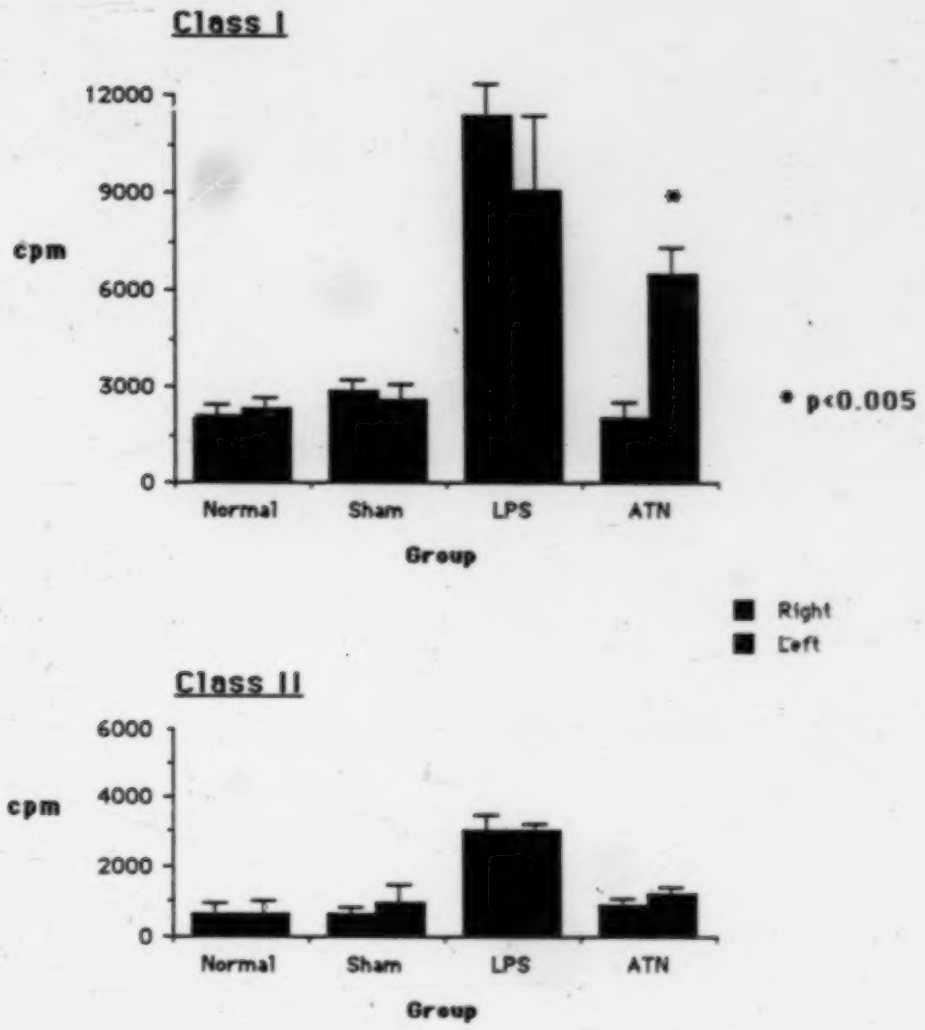

Figure 4: MHC Expression on Day 3 With 60' Clamping

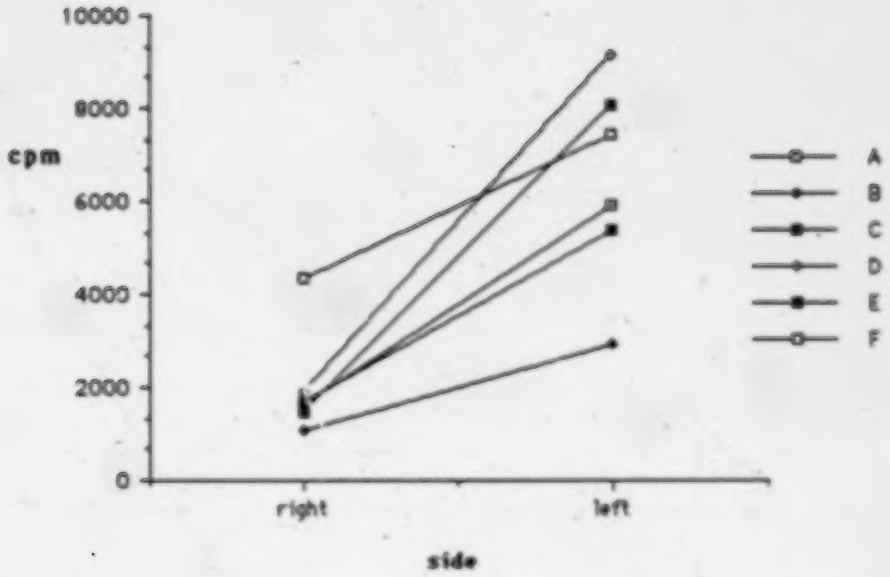

Figure 5: Class I Expression on Day 3

Plate 7a Control (right)

Plate 7b Ischemic (left)

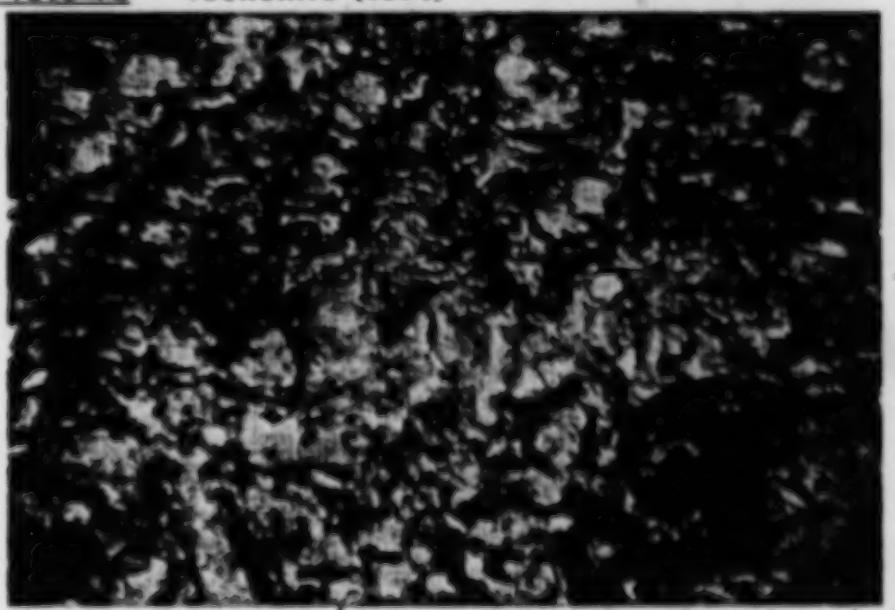

Plate 7: Immunoperoxidase stain for class I on day 3 in ischemic and control kidney. Note tubular staining on ischemic side (25x)

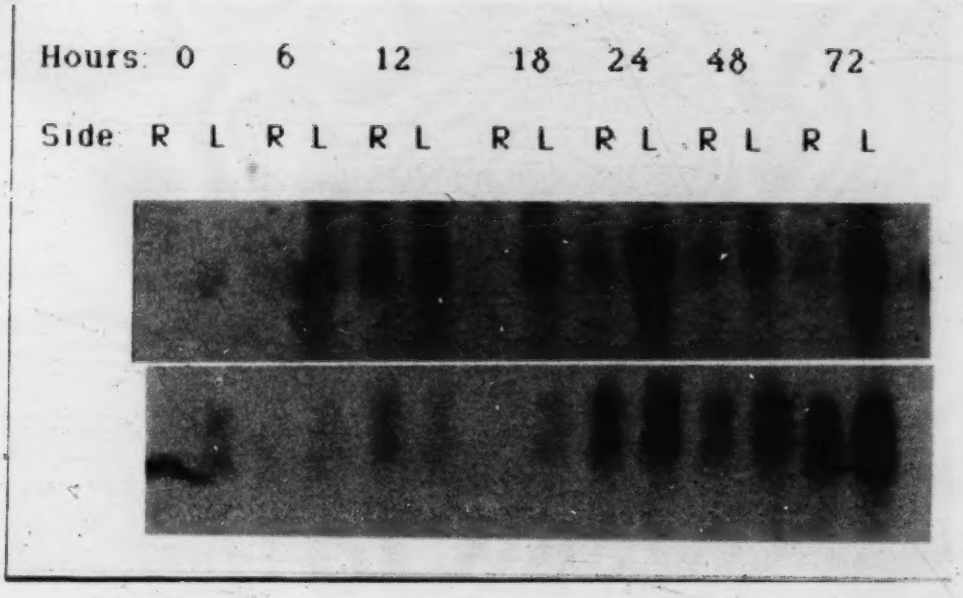

Plate 8: **Northern Blot of early ATN Kidneys.**

Upper probe is c-myc, lower is β_2 microglobulin

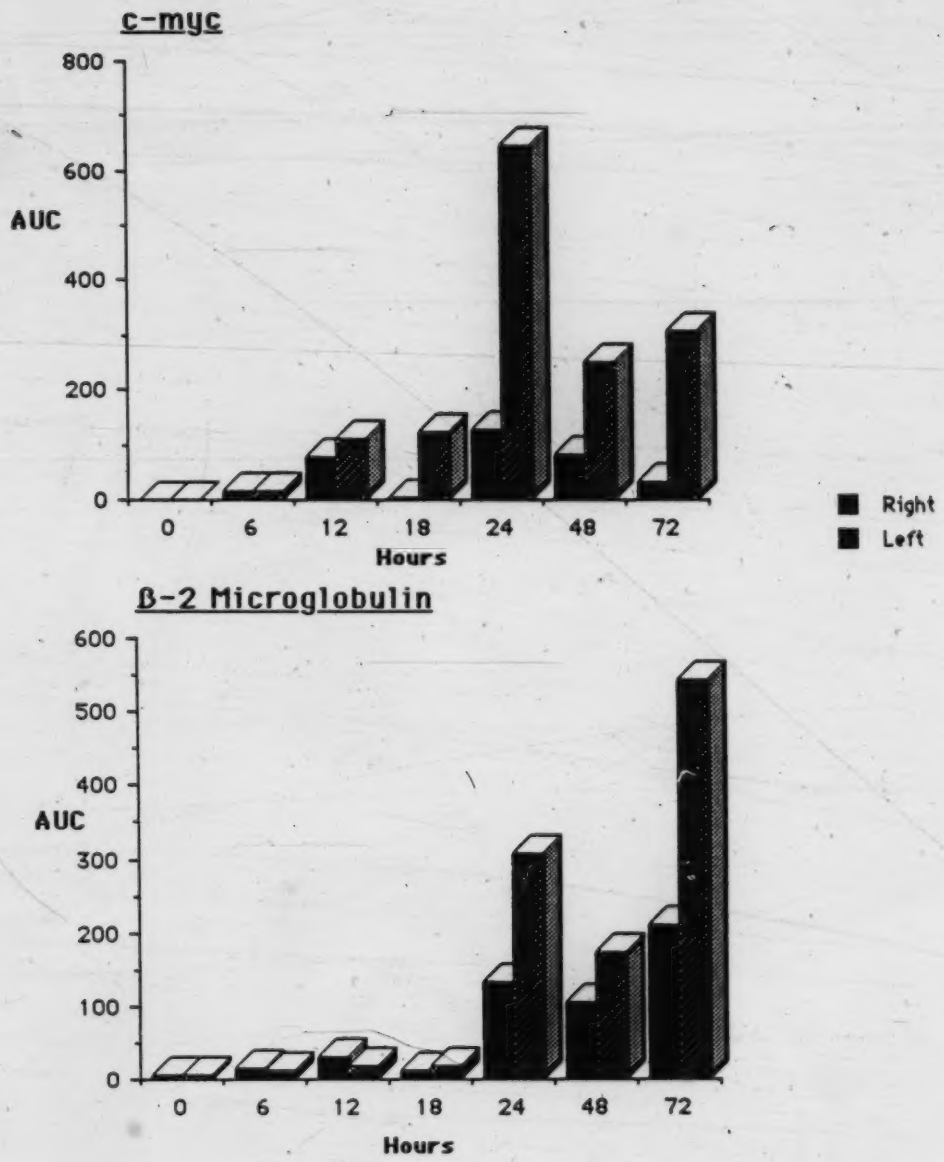

Figure 6: **Gene Activation in Early Ischemic ATN by Densitometry**

(AUC = Area under the curve)

The β_2 microglobulin mRNA was increased in the ischemic kidney at 24 hours, peaking by 72 hours. This mirrored the changes at the antigen product level but preceded it by 48 hours. Figure 6 shows the data quantitatively. Note that the 2-3 fold increase in the left over the right values was similar to that found at the product level by RIA. As well, MHC gene activation was preceded by c-myc activation by 12 to 24 hours.

It is standard practice to probe a northern blot with actin to ensure that each well of the gel had an equal quantity of RNA. This is based on the assumption that all cells produce an equivalent amount of this structural protein. In all the ATN blots probed however, the lanes from the ischemic kidneys consistently had higher levels of actin than in the nonischemic controls. This was true even when more RNA was intentionally loaded into the control wells. Because of this, the only data that supports the equivalence of the wells in these northerns is the ethidium bromide picture of the gel and the optical density readings performed on the samples before dilution.

Class I Tubular Induction Resolves on Day 5

As seen in figure 7, the class I tubular induction present in the ischemic kidney on day 3 resolved by day 5. Class II began to increase in the day 5 ischemic kidneys but statistical

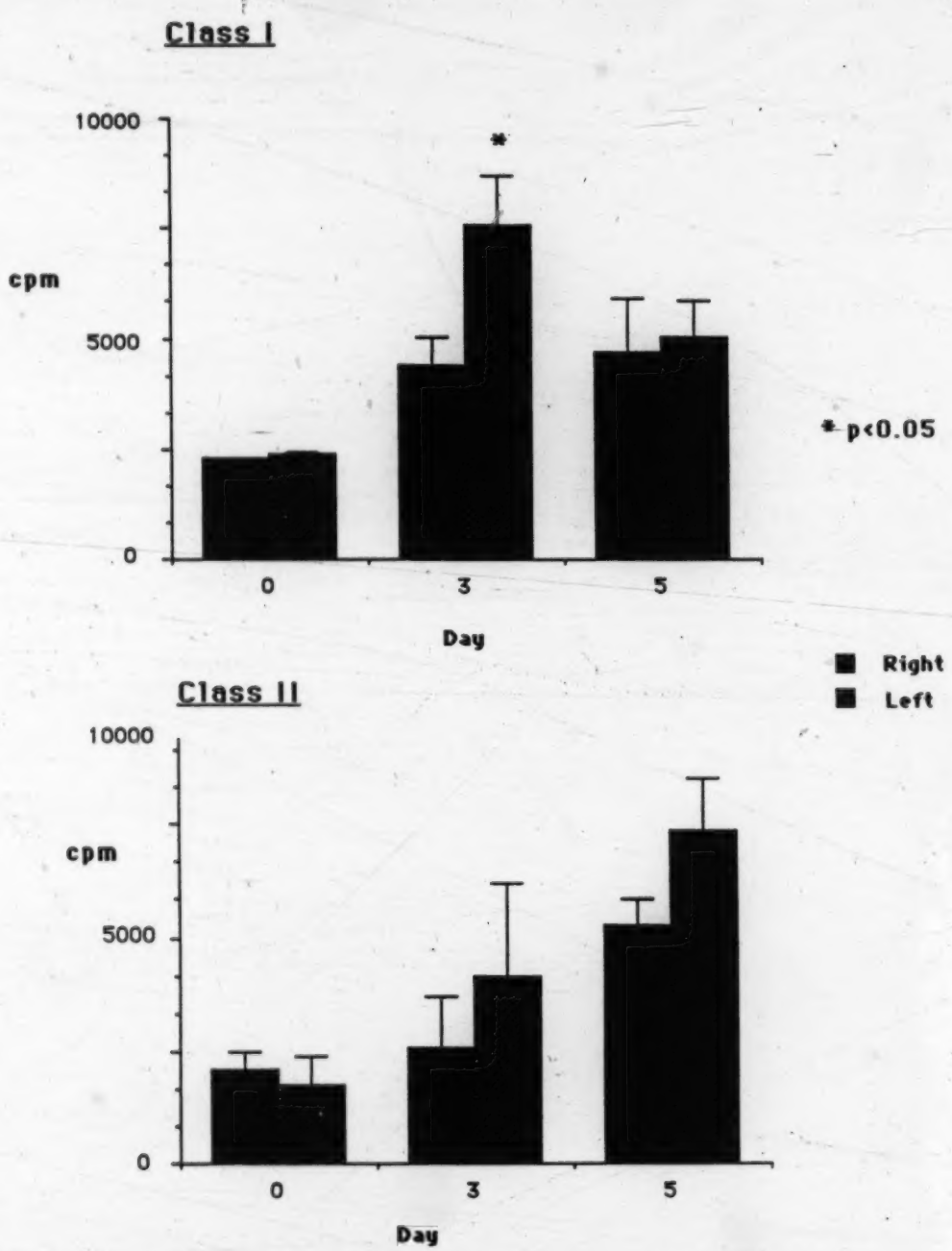

Figure 7: MHC Expression on Days 3 and 5

significance was not reached. Immunoperoxidase staining showed minimal differences between the day 5 kidneys.

Effect of Cyclosporine on Early Class I Induction

Cyclosporine given orally at a dose of 100 mg/kg daily prevented the tubular increase of class I on day 3 following surgery in 2 successive experiments. As seen in plate 9, the histologic changes indicative of cyclosporine renal toxicity (pale tubular cytoplasm with vacuolization) coexisted with the tubular necrosis and dilatation of ATN. This shows that the cyclosporine treatment did not prevent class I induction by preventing ATN. A representative RIA is shown in figure 8. In this experiment, LPS served as the positive control while LPS + cyclosporine served as a biological test of the drug's efficacy. Note that cyclosporine only partially blocked the LPS effect at the dose used. The ability of cyclosporine to prevent the ischemic MHC induction is seen in figure 9 where only 1 of 7 animals showed greater class I expression in the left kidney as compared to the right.

Cyclosporine exerted its effect at the mRNA level as seen on the northern in plate 10. Both right and left kidneys clearly show decreased transcription of the β_2 microglobulin gene. Cellular activation was also prevented as evidenced by the prevention of c-myc expression as well.

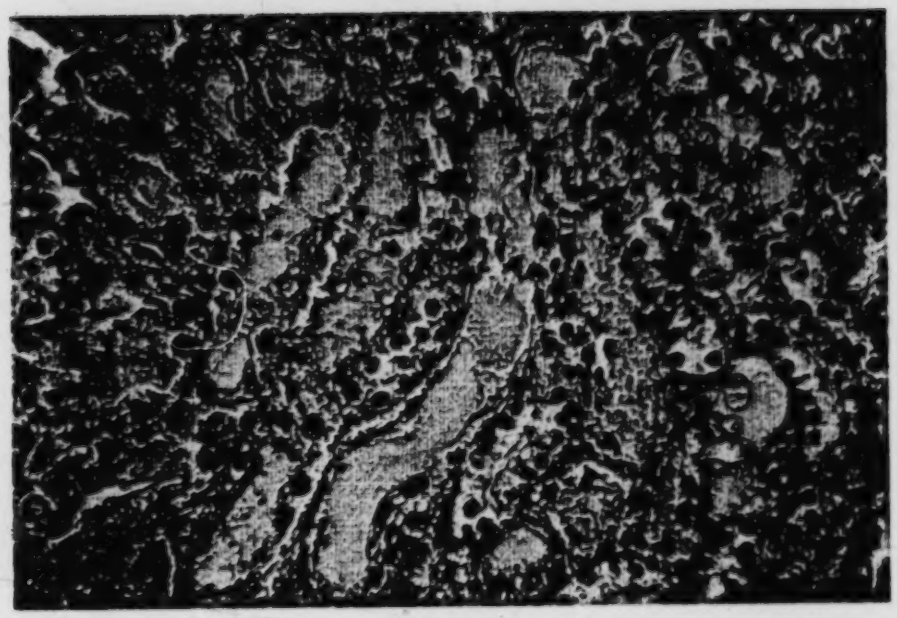

Plate 9: Photomicrograph of ischemic kidney from mouse receiving Cyclosporine (H+E stain, 25x)

Note vacuolization and cytoplasmic pallor of tubular cells

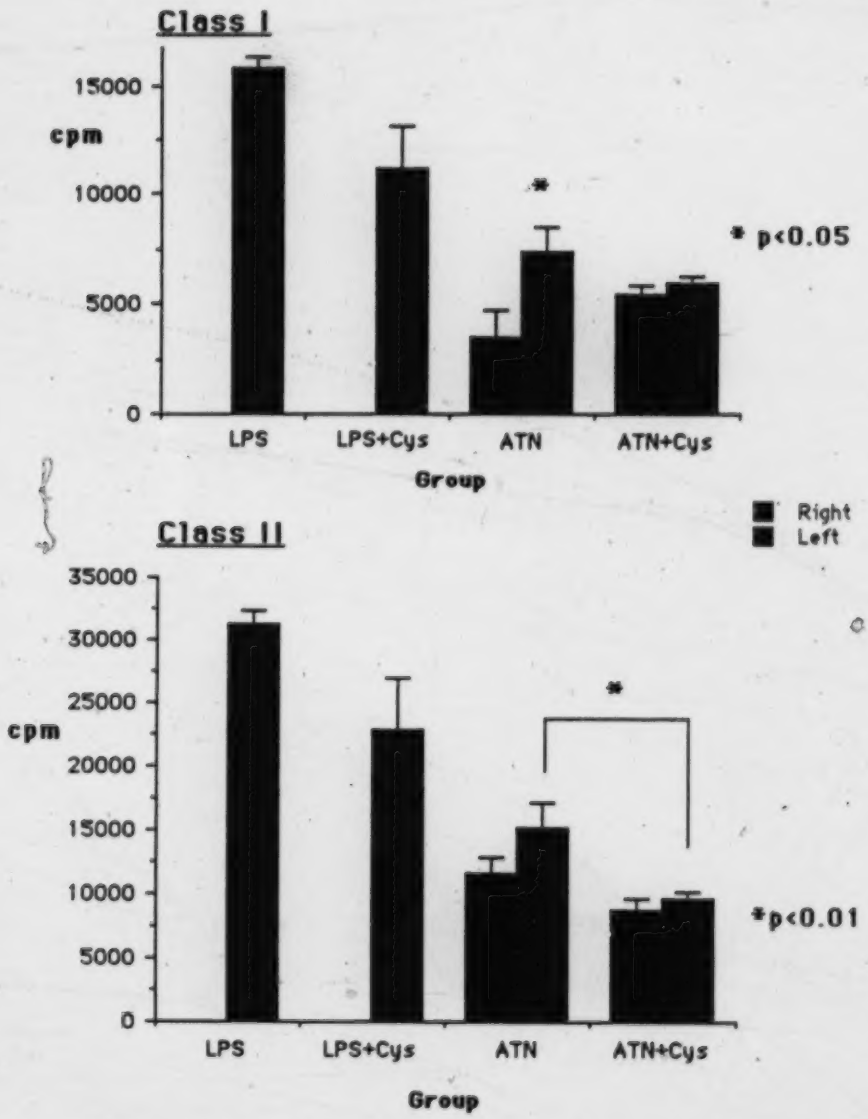

Figure 8: Effect of Cyclosporine on ATN at Day 3

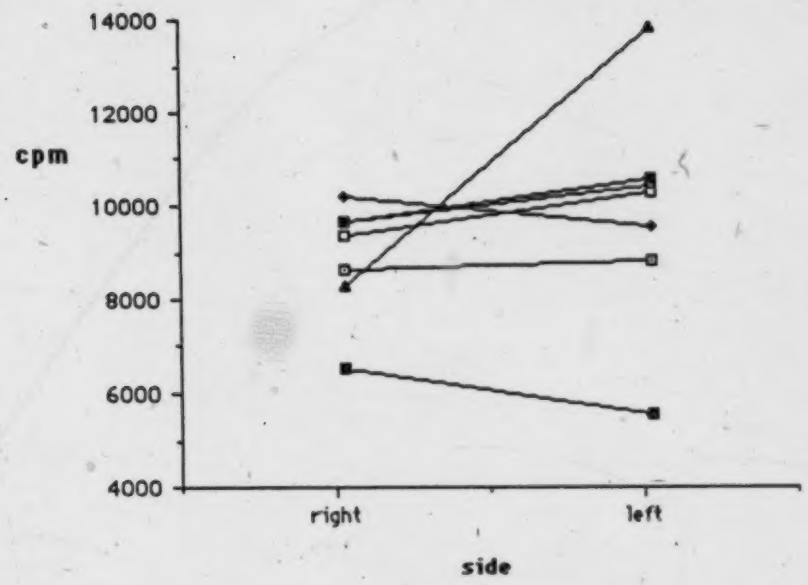

Figure 9: Class I for ATN + Cyclosporine

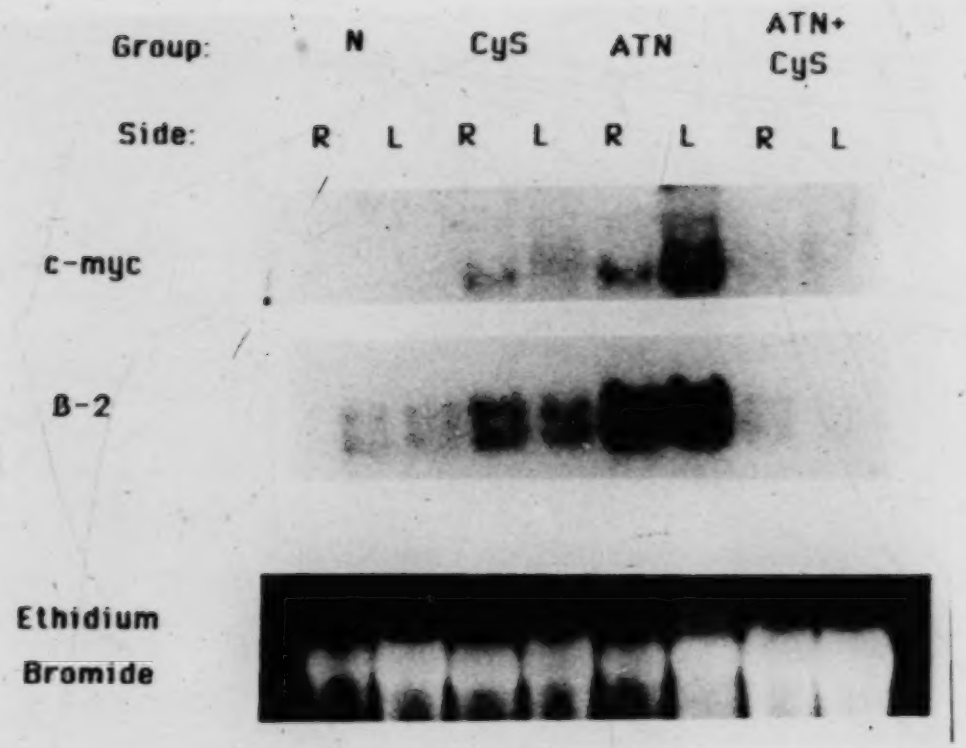

Plate 10: **Northern blot of ATN Kidneys With or Without Cyclosporine**

(N = Normal, Cys = Cyclosporine)

Effect of anti-IFN-γ on Early Class I Induction

Anti-IFN γ antibody given i.p. before, during and after surgery was able to prevent the tubular class I induction on day 3. As seen in figures 10 and 11, the antibody reduced the MHC induction produced by LPS and prevented the induction seen in ATN. While the ischemic kidneys in the ATN+antibody group did have a class I expression higher than the controls, this was significant only at the p= 0.10 level. All ischemic kidneys in this group had ATN on histology and no difference in immunoperoxidase staining between the sides (data not shown).

Plate 11 shows a northern for $ß_2$ microglobulin performed on day 3 kidneys after ATN with or without cyclosporine or anti-IFN γ . The mRNA was reduced in the cyclosporine group, while there was still an increase in the ischemic ATN + anti-IFN γ kidneys, though not as much as with ATN alone. The densitometric quantification is presented in figure 12.

Changes in MHC Expression: 1 to 3 Week Time Points

When the experiment was extended to include days 7, 14 and 21, MHC products were again increased. As figure 13 shows, the ischemically damaged left kidneys had increased class I expression from days 7 to 14. In contrast to the day 3

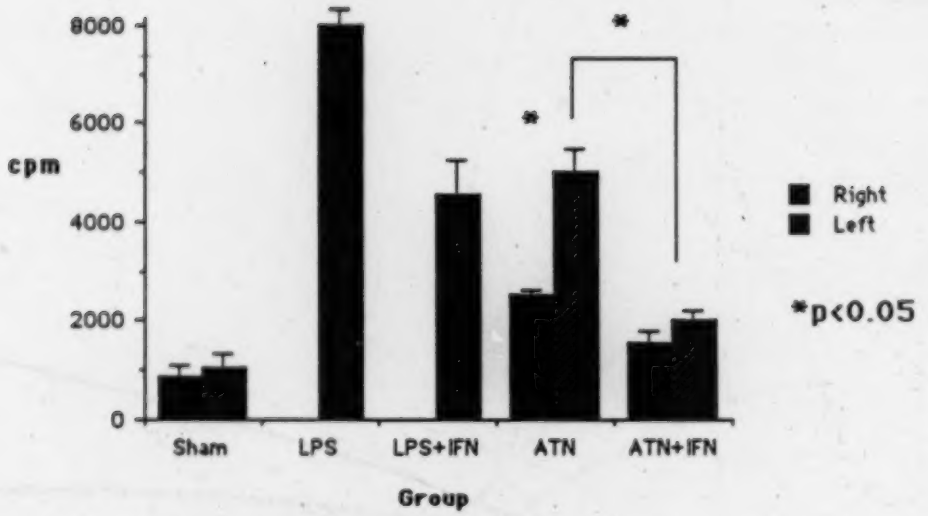

Figure 10: Effect of anti-IFN gamma on class I Expression on Day 3

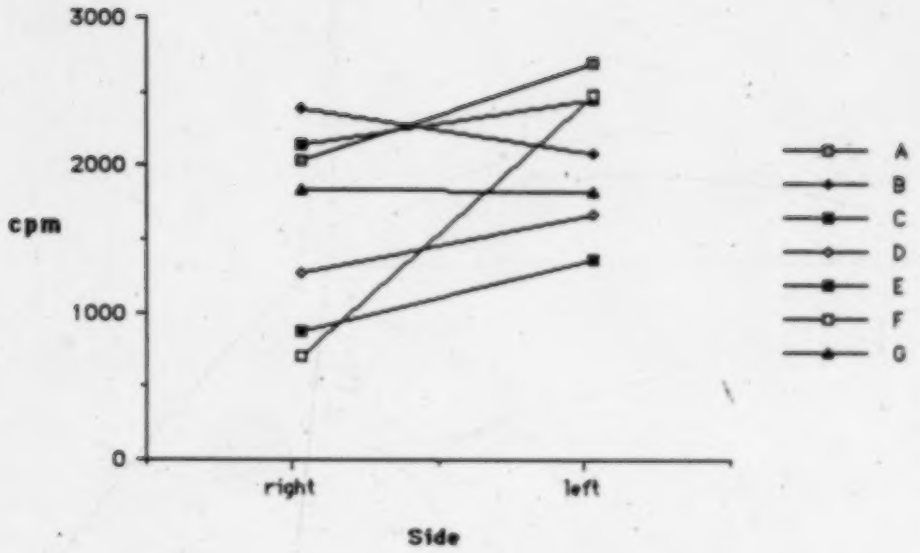

Figure 11: Class I for ATN + anti-IFN gamma

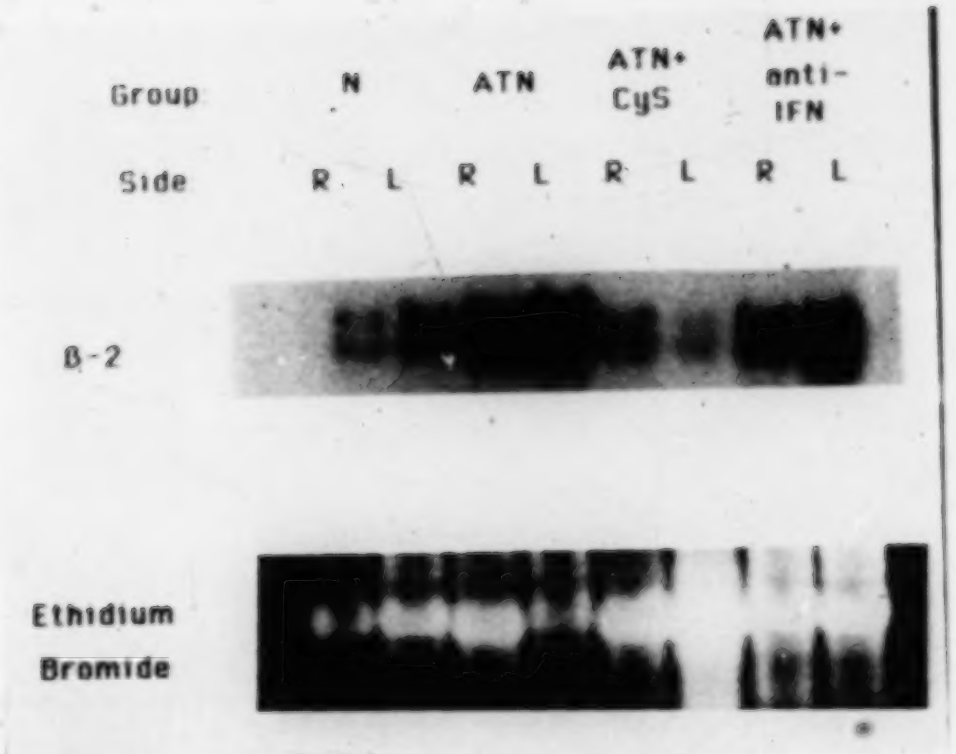

Plate 11: **Northern blot of ATN Kidneys With or Without Cyclosporine or anti-IFN gamma antibody**

(N = Normal, Cys = Cyclosporine IFN = Interferon)

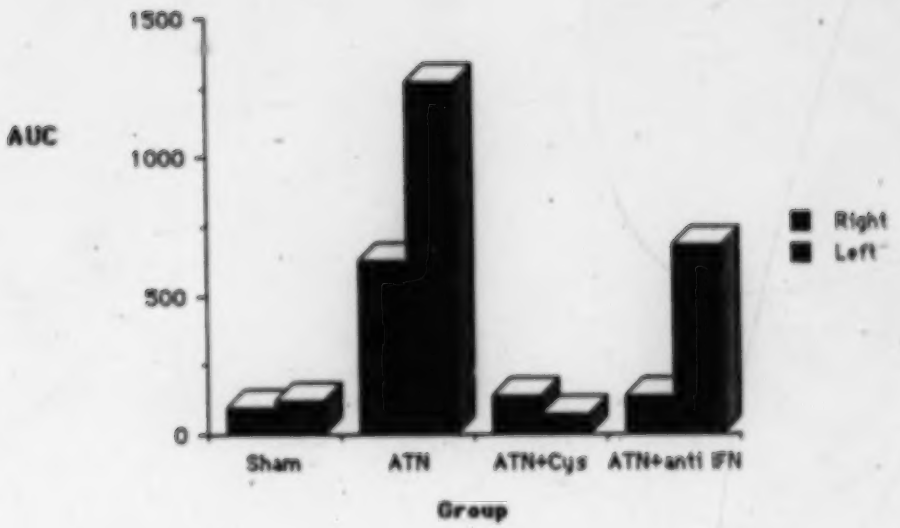

Figure 12: Densitometry of Northern Comparing ATN Alone With ATN + Cyclosporine or anti-IFN gamma antibody for Class I

(AUC = Area under the curve)

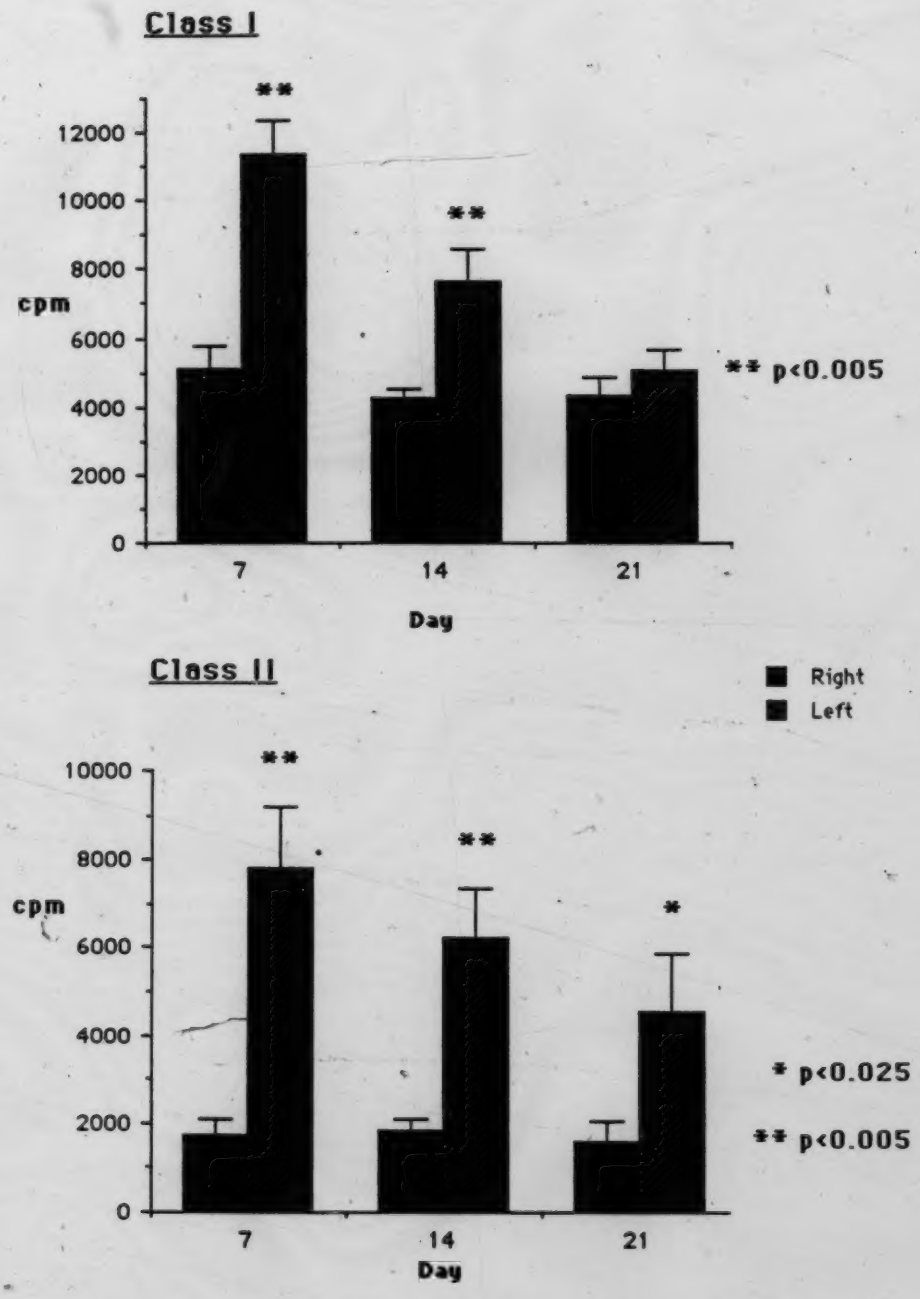

Figure 13: MHC Expression For Days 7 to 21

experiments, class II was increased from 2-4 times in the ischemic kidney over the control. This increase persisted beyond day 21 to a small group of day 35 animals. The class II data for day 14 is shown by animal in figure 14. Of interest is the observation that the two animals without class II induction exhibited a minimal infiltrate in the ischemic kidney. Beyond that, there was no correlation between histologic features and degree of MHC induction for either class.

The immunoperoxidase staining for the experiments is summarized in table 2. As can be seen, the class I staining so prominently tubular after day 3 was primarily interstitial from days 7 to 21. The class II staining, first evident on day 7, was primarily interstitial. In kidneys with a large inflammatory infiltrate (6/26), the class II stained tubular cells as well (plate 12). No Thy1.2 positive cells were seen until day 7, where a progressive growth of an inflammatory population began. Of interest in day 14 to 21 specimens, the interstitial staining for Thy1.2 was so pervasive that the appearance could not be distinguished from that of tubular staining (plate 13).

The northerns on kidneys from 1 to 21 days are reproduced in plate 14. Both β_2 microglobulin and Aα (class II) mirrored the increases seen in the left kidneys in the RIA's but again occured about 24-48 hours prior to the antigen expression (note the large peaks at day 5) (figure 15). c-myc expression peaked at day 1, slowly decreased until day 7 and was then not

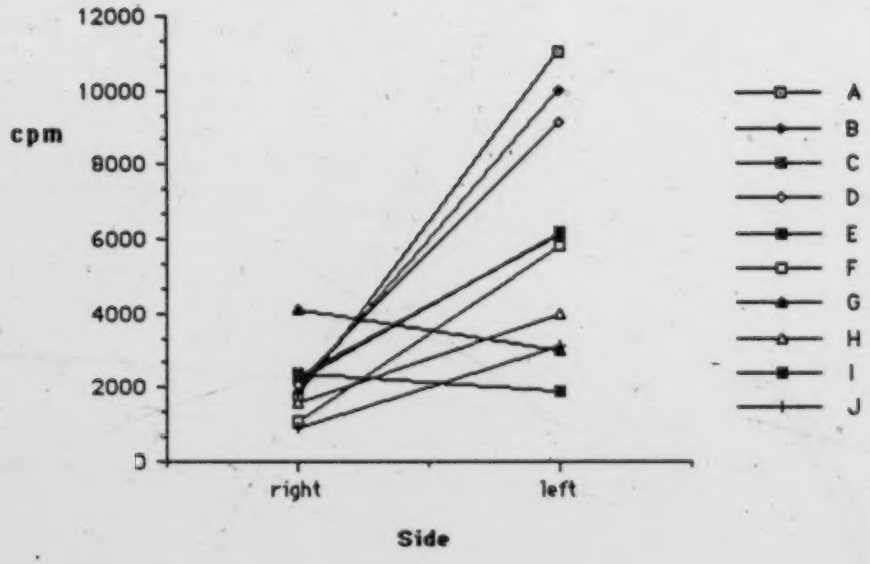

Figure 14: Class II on Day 14

Day	Class I		Class II		Thy1.2	
	Tubular	Int	Tubular	Int	Tubular	Int
1	+/-	-	-	-	-	-
3	+++	+	-	+/-	-	-
5	+/-	+/-	-	+/-	-	-
7	+	++	++	+++	-	++
14	-	++	+	+++	+	++
21	-	+	+/-	+++	+	++

Table 2: Summary of Immunoperoxidase Staining

(Int = Interstitial)

Plate 12a Note primarily interstitial staining

Plate 12b Note tubular and interstitial staining

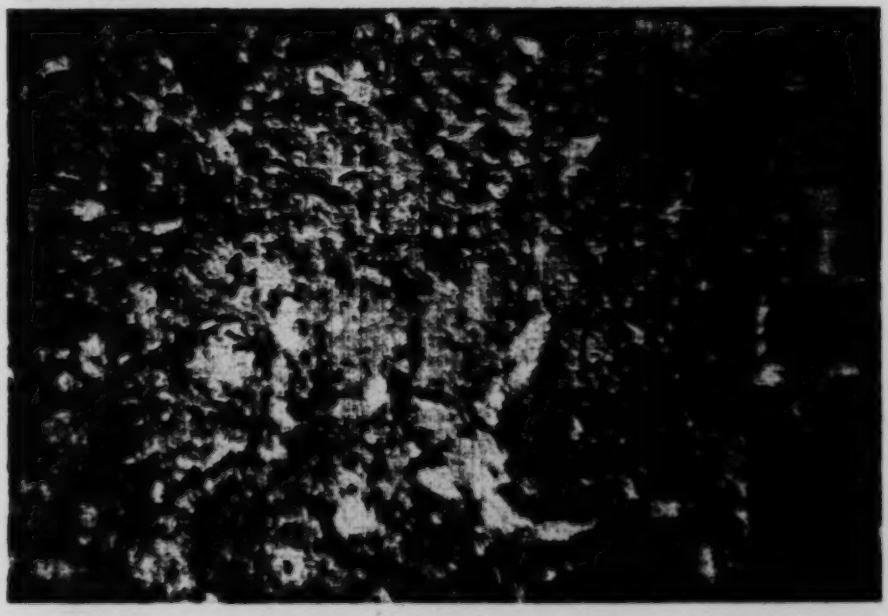

Plate 12: Immunoperoxidase staining for class II on day 7. Note staining of interstitial +/- tubular cells

Plate 13a Day 14 (10x)

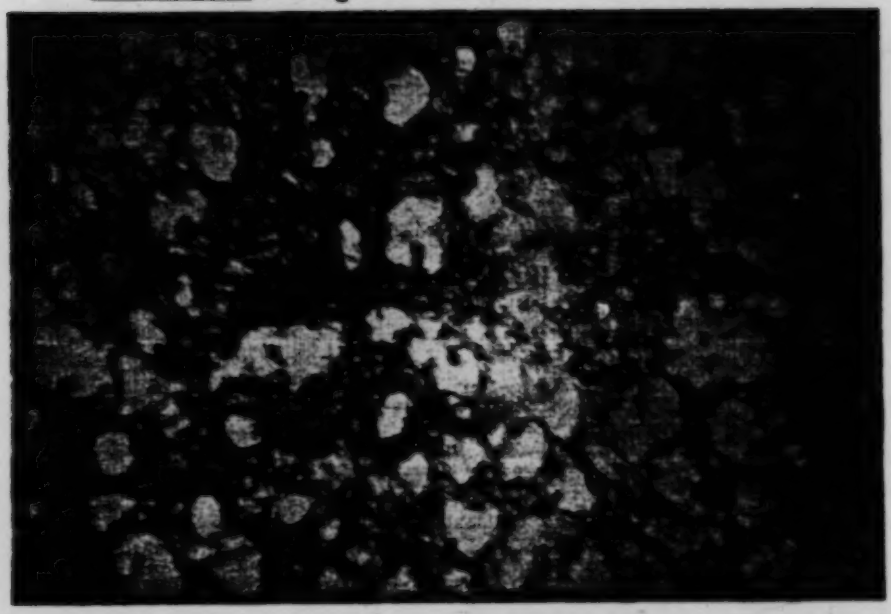

Plate 13b Day 21 (25x)

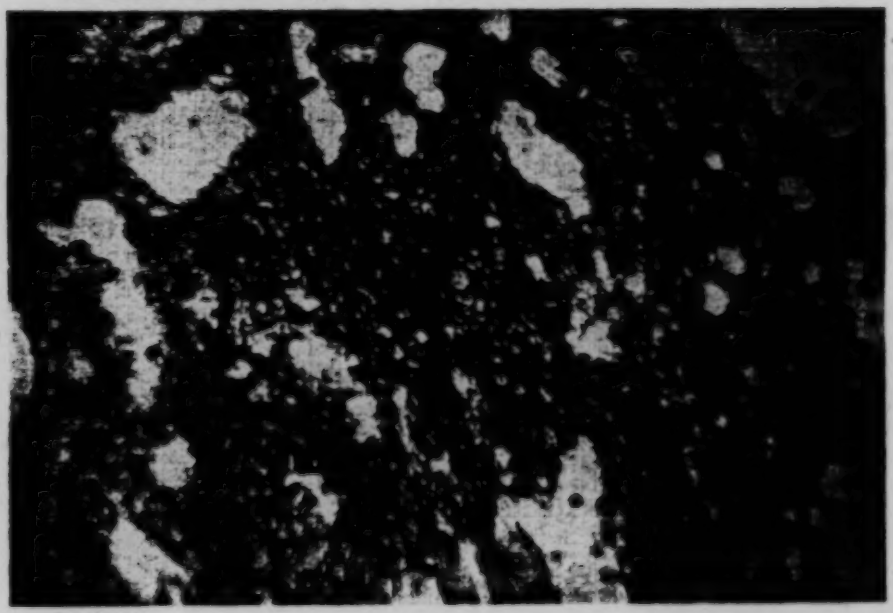

Plate 13: Immunoperoxidase staining for Thy1.2 on days 14 and 21.

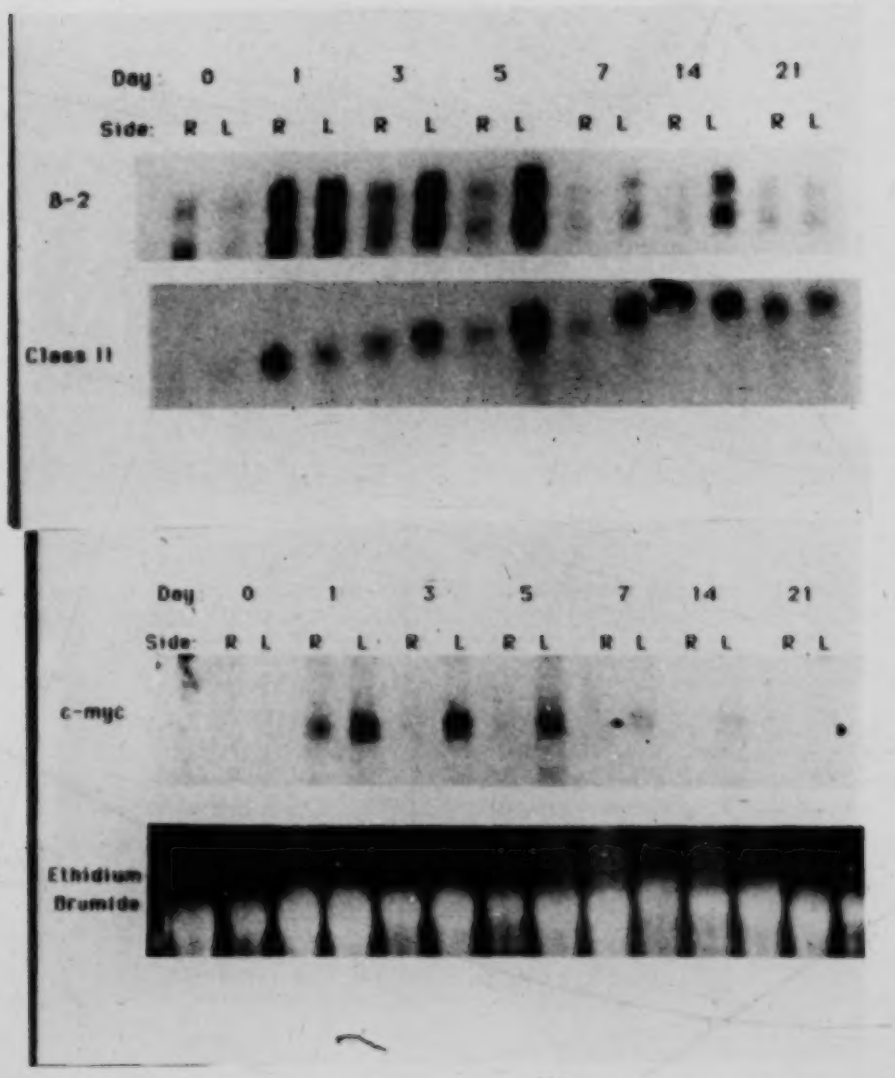

Plate 14: Northern blot of ATN Kidneys From Day 1 to Day 21

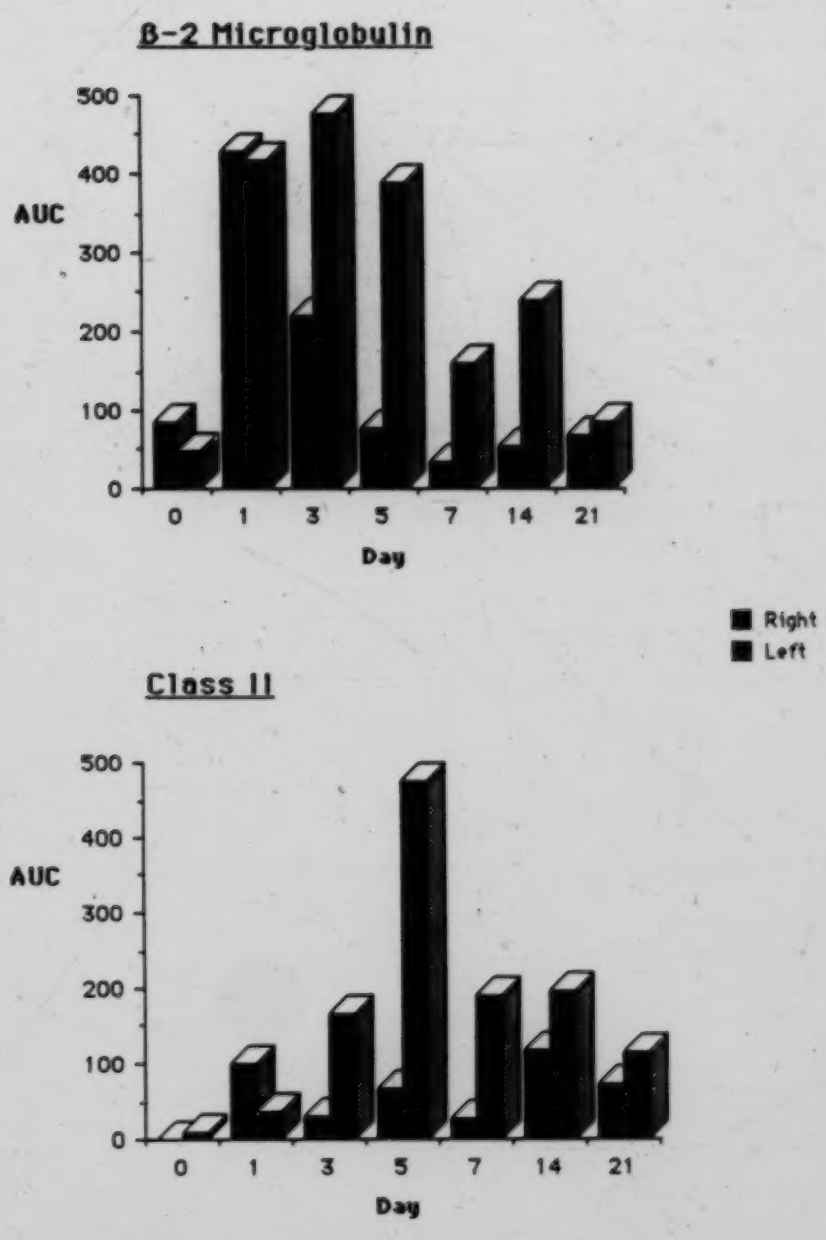

Figure 15: Densitometry of Northerns for MHC in Ischemic ATN

(AUC = Area Under the Curve)

detectable. c-myc was seen at low levels in the right kidney only at day 1 (figure 16).

Effect of Cyclosporine on Day 7

The results of an experiment comparing ATN and ATN + cyclosporine on day 7 are presented in figure 17. The cyclosporine had no effect on either class I nor class II induction in the left ischemic kidneys. This was born out at the mRNA level and with immunoperoxidase staining (data not shown). This was in contrast to the day 3 class I increase, which was completely blocked by cyclosporine.

Summary of 60' Clamping Experiments

In order to compare the results from multiple RIA experiments, the cpm results were divided in each group by the counts for the control tissue. The resulting combined results are shown in figure 18. Note that the statistics were non-parametric for this analysis. For class I, the left kidney became induced transiently on day 3 and then again from days 7 to 14. For class II, induction began on day 7 and persisted until day 35 (the lack of statistical significance on day 35 was due to the small numbers in that group). Table 2 summarizes the histochemical staining.

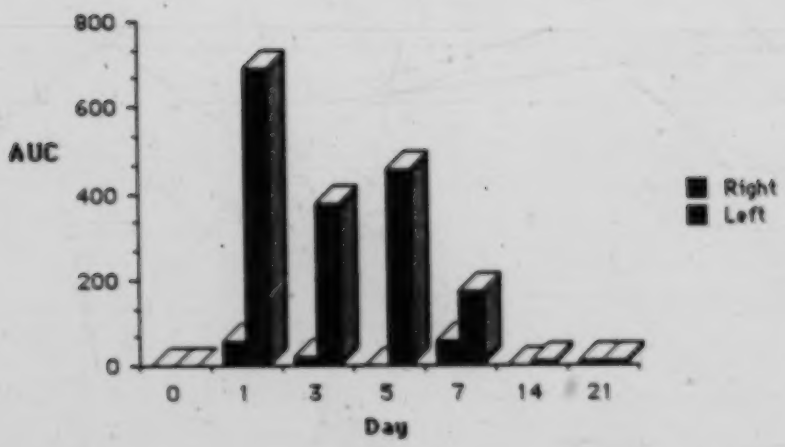

Figure 16: Densitometry of c-myc Expression

(AUC = Area under the curve)

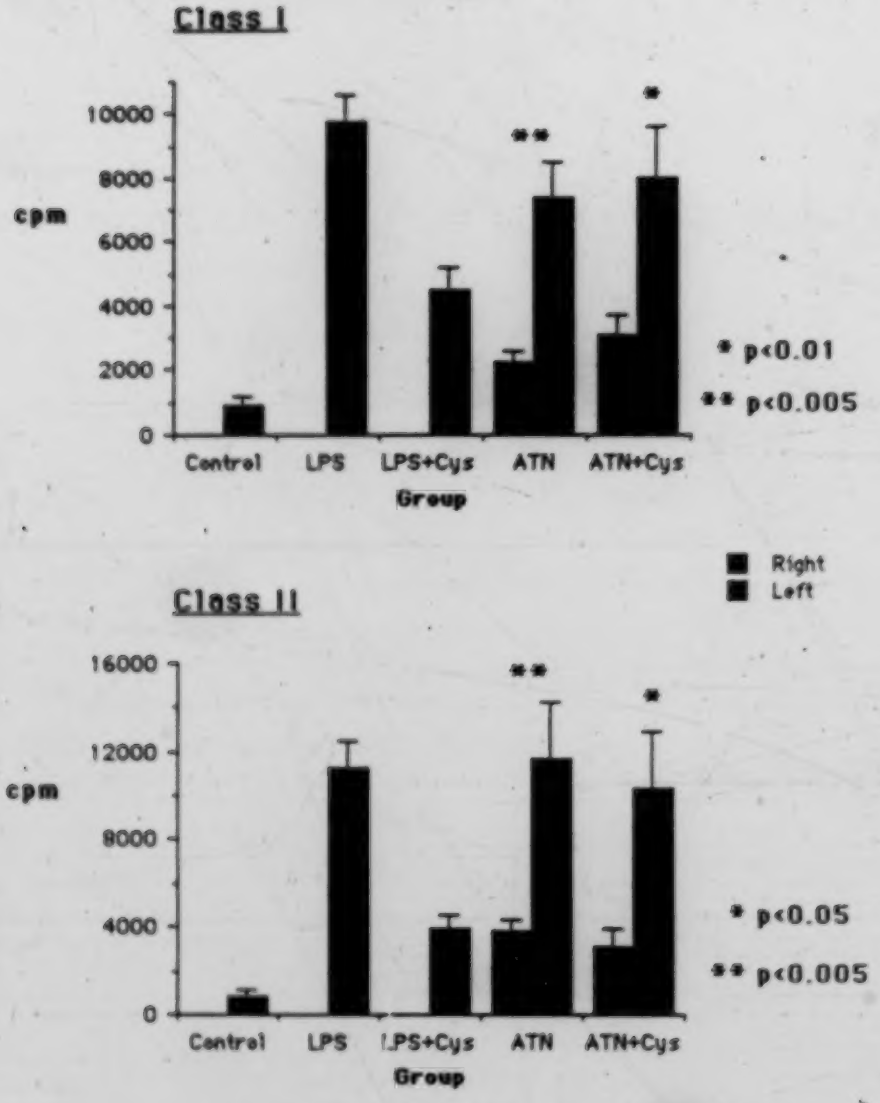

Figure 17: Effect of Cyclosporine on MHC at Day 7

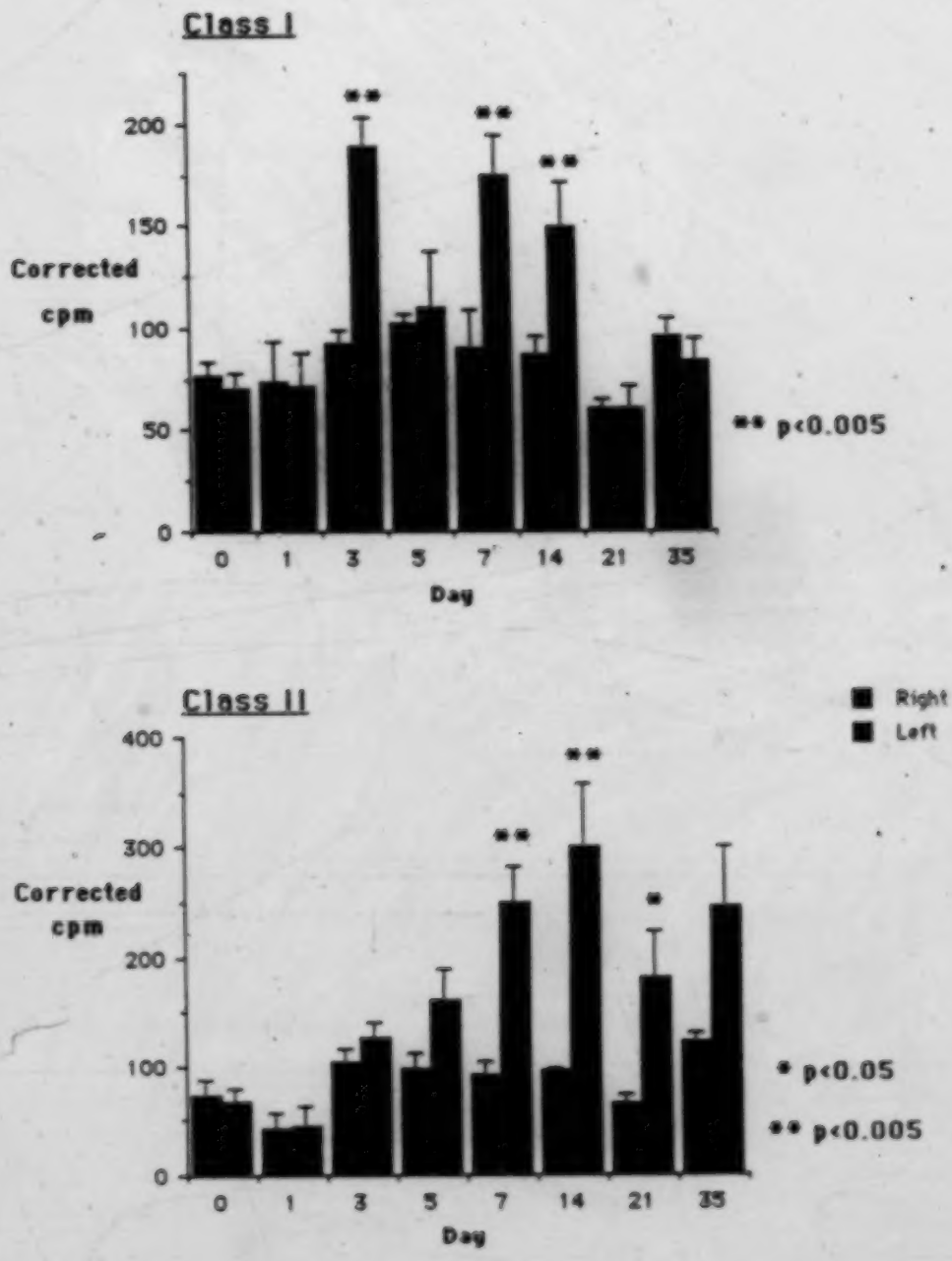

Figure 18: Combined RIA Data For MHC

Experiments in Nude Mice

An experiment was done with nude mice (balb/c background) with 60' clamping at days 3 and 7. Because of an ongoing technical problem with the antibodies for this strain in our laboratory, an RIA could not be done by the time of this publication. The northern however showed a suprising finding. As seen in plate 15, both class I and class II were increased on both days 3 and 7. This is quantified in figure 19. There was a 2 to 3 fold increase in both classes on both day 3 and 7.

Immunoperoxidase staining showed that the class I increase was tubular on day 3 and both tubular and interstitial on day 7. Class II stained the tubules in 2/4 of the day 3 animals. Surprisingly, the 2 day 3 kidneys that stained for class II also stained positively for Thy 1.2 on interstitial cells. Class II was seen in the tubules on day 7 in both the right and left kidneys, raising the possibility of a systemic infection in this group.

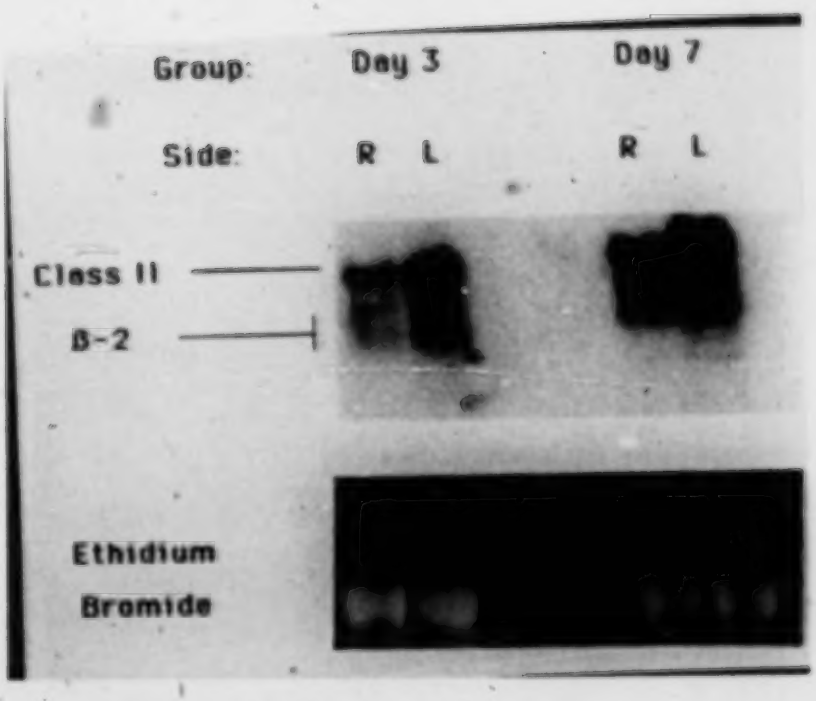

Plate 15: Northern Blot for B-2 and Class II on kidneys from nude mice on days 3 and 7.

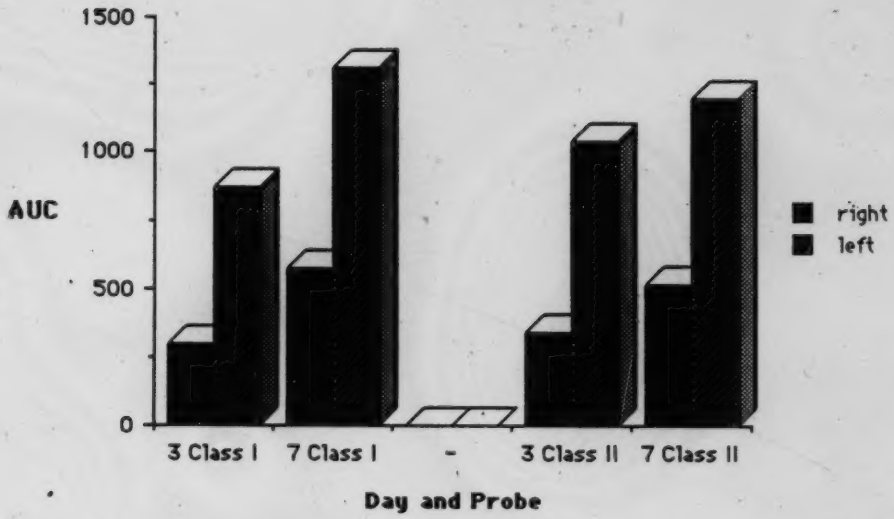

Figure 19: Densitometry of Northern for MHC in Nude Mice with ATN on Days 3 and 7

(AUC = Area under the curve)

Discussion

Unilateral Renal Ischemia as a Model for Tx ATN

While the mouse has seldom been used for studies of unilateral ATN[134], it appears to be as reliable as the more commonly studied rat and rabbit models. The primary advantage of its use is the greater availability of monoclonal antibodies and molecular probes developed in the mouse.

The advantage of using a unilateral model was the presence of a normal contralateral kidney. This controlled for other effects of surgery, systemic release of cytokines and the natural inter-animal variation in MHC expression commonly seen in animals not kept in a sterile environment.

The histology shows that 60' warm ischemia does produce reliable ATN. Interestingly, there was no evidence of vessel thrombosis, even after 5 weeks, despite the fact that heparinization or flushing was not used. Tx ATN is different from native ATN in that the degree of tubular necrosis is more uniform rather than focal, and that an inflammatory infiltrate is prominent[2]. The fact that this model of "native" ATN behaves more like Tx ATN probably reflects the common etiology of complete cessation of blood flow to the kidney. In ischemic native ATN from profound shock, there is still some degree of perfusion and oxygenation, which explains why animal shock models of ATN

are so difficult to produce. The main limitation of this model in its extrapolation to the allograft setting is the nature of the inflammatory cells: isogenic in this model and allogenic in transplants.

Normal Paired Kidneys Have Equal MHC Expression

The finding that MHC expression is equal in both kidneys for normal animals, for those undergoing a laparotomy and for those with 15' of renal pedicle clamping is not unexpected, but new in the literature. It serves as the principal control to ensure that changes from the ATN were not due to another variable in the operation, in particular, vascular damage by the clamp with subsequent thrombosis. This was further supported by the finding that lesser degrees of ATN as assessed histologically correlated with a lower degree of class I induction in the day 3 animals.

Early Changes in Cell Activation

Histologic evidence of ischemic damage was first seen 6 hours after reperfusion, increased at 18 hours, and peaked from 24 to 72 hours. The cellular proto-oncogene c-myc had minimal expression at 6 hours, increased at 12 hours and peaked at 24 hours. While the exact function of c-myc is unknown, it produces a protein that binds to DNA in the nucleus and influences gene transcription[119-121]. While c-myc induction has been reported in

other forms of ischemic injury[126], this is the first report of its increase in ischemic ATN. The low level of c-myc expression seen in the control kidney at 12 hours may have been in response to a growth factor signal released when the damaged kidney became temporarily nonfunctional.

Whatever the role of c-myc in cell activation and repair, it cannot be used as a pure measure of ATN. Cyclosporine blocked the expression of the proto-oncogene but did not affect the degree or recovery of ATN as seen histologically. Either the c-myc product is not associated with cellular activation and repair in an ischemic kidney or else the process can occur in its absence.

Increased Class I on Tubular Epithelium on Day 3

MHC class I antigen expression was increased transiently but consistently on tubular epithelial cells 3 days following surgery. This occurred in the absence of a demonstrable infiltrate or changes in class II expression. This increase was blocked completely by cyclosporine and decreased to non-statistical significance by anti-IFN γ antibody. IFN γ is the most potent inducer of MHC antigens *in vivo* but usually increases the expression of both class I and II together[77-82,87]. The lack of class II expression in this apparently IFN γ dependent response could be explained in 2 ways. First, IFN γ appears to affect class I preferentially, particularly at low doses (unpublished observation). The low levels of cytokine produced locally in the

ischemic kidney may have been insufficient to induce detectable class II antigen (although there was a small increase in class II mRNA). Second, there may have been a second cytokine which prevented the class II increase. The type I interferons (α/β) are known to increase class I but decrease class II when present in combination with IFN γ[87]. Their release could explain why class II was unaffected but class I still partially increased when IFN γ was blocked. Certainly, most cytokines are released in groups or "cassettes" *in vivo*, so the presence of other mediators of MHC unmeasured in these experiments would not be unexpected.

The ability of cyclosporine to block the class I tubular increase may have been through its ability to prevent IFN γ release by blocking IL-2 production[144]. Other mechanisms may be involved however, as cyclosporine has effects independent of cytokines (as evidenced by its toxicity at high doses)[89,90]. Of particular interest is the recent finding that cyclophilin, the cyclosporine binding protein in the cell, is identical to an enzyme which catalyzes the *cis-trans* folding of certain proteins[91,92]. Thus, cyclosporine may block other important metabolic pathways which could influence MHC regulation.

The fact that this mechanism is cytokine dependent raises the question of their origin. In allograft rejection, infiltrating T cells are the primary source of IFN γ. They would be an unlikely source in our model because of the absence of a gross infiltrate or any Thy 1.2 positive cells at the day 3 time point. Furthermore,

the class I increase was intensified in the nude mice, which are supposed to be devoid of alloreactive T cells. One possible source could be the marrow derived cells occurring naturally in the interstitium, the so called dendritic cells. Indirect evidence for their activation is seen histologically, with interstitial proliferation in the absence of an infiltrate seen from days 5 to 7. Another source could be natural killer cells which are known to secrete IFN γ . Their presence in large numbers in athymic mice could explain both the increased MHC expression and the Thy1.2 staining seen in the nudes, as a population of Thy1.2 positive natural killer cells has been characterized[145,146].

Increased MHC Expression on Day 7 and Beyond

Seven days after surgery, the tubular obstruction was resolving, fewer dead tubular cells were seen and inflammatory cells accumulated in the interstitium. This interstitial inflammation is a feature of both experimental and human ATN[12-15]. Class I antigen expression peaked a second time on day 7 and was primarily interstitial. Class II expression, both interstitial and tubular, was first seen on day 7 and persisted up to day 35. The class II increase was associated with the presence of Thy1.2 positive cells in the interstitium. Again, changes in antigen product were mirrored and preceded by changes in mRNA. Unlike the MHC induction on day 3, the day 7 increase was unaffected by cyclosporine (because of this, anti-IFN γ was not tried).

One explanation for these findings is that the increased MHC expression quantified in the kidney as a whole is simply reflecting the change in cellular population, namely the class I and II positive inflammatory cells. While this could explain the interstitial class I increase, and its resistence to cyclosporine, there was a definite tubular class II expression seen in some of the specimens. Either this tubular induction was caused by a cytokine not blocked by cyclosporine (such as tumour necrosis factor[87]), or the class II gene was expressed as part of the reparative response to ischemia.

Relevance to Transplant ATN and Rejection

The increase of MHC products in ischemic ATN may help explain a well described but poorly understood clinical phenomenon. Since 1973, it has been reported that transplant ATN (as diagnosed clinically by initial nonfunction and/or the need for post-transplant dialysis) is associated with a higher incidence of ultimate graft loss by rejection[17]. As summarized in the literature review, large multi-centre studies have consistently found this relationship[19,25-27,36].

The finding of increased MHC expression in this experimental model suggests a possible mechanism for the detrimental effects of transplant ATN. First, the early increased tubular class I expression could increase the response of $CD8^+$

cytotoxic T cells[63-65], thereby hastening allograft destruction. It also suggests a mechanism for the combined ATN and early acute rejection pattern sometimes seen in cases of primary graft nonfunction. Second, the increased class II expression, especially of tubular cells seen at 1 week in the model, could increase the response of $CD4^+$ helper T cells, thereby activating the cascade of immunological rejection[66]. Finally, the accumulation of the interstitial infiltrate seen in pure ATN suggests the presence of a local signal which attracts inflammatory cells. If this signal also exists in transplant ATN (above and beyond the allograft response), it could hasten the immunologic response by attracting more T cells.

Most modern protocols of maintenance immunosuppression in renal transplantation include cyclosporine[32,33,36]. The fact that the early class I induction was blocked by cyclosporine does not rule it out as a possible mechanism in these patients. The dose used in the mice (100 mg/kg per day) is that known to block cytokine production. This was borne out by its ability to block the MHC induction from LPS. In humans however, it has not yet been determined whether the tissue concentrations achieved by cyclosporine are sufficient to block the effects of cytokines in the presence of rejection or ischemia. While this is one of the suggested mechanisms of cyclosporine's immunosuppressive effect[144], it has yet to be proven clinically.

Suggestions For Further Study

The finding that native ischemic ATN causes increased MHC expression is new and may help explain the detrimental effects of clinical Tx ATN. The next step would be to determine whether this effect is also seen in a vascularized allograft model. The natural extension of these experiments would be to perform renal transplantation in the mouse, varying the warm ischemia times, and comparing MHC expression in the donor kidneys for both isografts and allografts. While the technology for mouse renal transplantation exists, it is notoriously difficult and done only in a few centres[102]. The alternative would be to attempt to reproduce the unilateral ischemic model in the rat and then perform a rat renal allograft experiment along similar lines.

An alternate line of investigation could involve the testing of human renal biopsies, comparing degree of ischemic damage with MHC expression in cases of both native and transplant ATN. The use of fine needle aspiration techniques is making routine post-transplant biopsies safer and in many centres a routine part of clinical follow up, and could provide a large amount of material for a prospective study.

It is hoped that a better understanding of the effects of ischemic damage on the immunogenicity of renal allografts and its prevention will allow better graft and patient survival for recipients of these kidneys in the future.

References

1. Hou SH, Bushinsky DA, Wish HB, Cohen JJ, Harrington JT. Hospital acquired renal insufficiency: A prospective study. Am J Med 74:243-248, 1983.

2. Solez KS. The pathology and pathogenesis of human "acute tubular necrosis". In: Acute renal failure: Correlation between morphology and function. Ed Solez K, Whelton A. Marcel Dekker, New York, 1984. pp 17-42.

3. Pegg DE. Organ Preservation. Surg Clin of N Am 1986; 66:617-632.

4. Burnier M, Schrier RW. Pathogenesis of acute renal failure. Adv Exp Med 1986; 212:3-13.

5. Osswald H, Schmitz HJ, Kemper R. Tissue content of adenosine, inosine and hypoxanthine in the rat kidney after ischemia and postischemic recirculation. Pfleugers Arch 1977; 371:45-49.

6. Kellogg EW, Fridovich I. Superoxide, hydrogen peroxide and singlet oxygen in lipid peroxidation by a xanthine oxidase system. J Biol Chem 1975; 250:8812-8817.

7. Fridovich I. The biology of oxygen radicals. Science 1978; 201:875-880.

8. McCord JM. Oxygen-derived free radicals in postischemic tissue injury. N Engl J Med 1985; 312:159-163.

9. Raytch RE, Bulkley GB. Free-radical-mediated postischemic reperfusion injury in the kidney. J Free Radical Biol Med 1987; 2:311-319.

10. Paller MS, Holdal JR, Ferris TF. Oxygen free radicals in ischemic acute renal failure in the rat. J Clin Invest 1984; 74:1156-1164.

11. Bayati A, Hellberg O, Kallskog O, Wolgast M. Effects of scavengers toward oxygen-free radicals in rat kidneys subjected to ischemic damage. Transplat Proc 1986; 18:54-85.

12. Solez KS, Kramer EC, Heptinstall RH. The pathology of acute renal failure. Leukocyte accumulation in the vasa recta. Am J Pathol 1974; 74:31a.

13. Solez KS, Morel-Maroger L, Sraer JD: The morphology of "acute tubular necrosis" in man: Analysis of 57 renal biopsies and a comparison with the glycerol model. Medicine 1979; 58:362-376.

14. Burdick JF, Beschorner WE, Smith WJ, McGraw D, Bender WL, Williams GM, Solez KS. Characteristics of early routine renal allograft biopsies. Transplantation 1984; 38:679-684.

15. Solez K, McGraw DJ, Beschorner WE, Kone C, Racusen LC, Whelton A, Burdick JF. Reflections on use of the renal biospy as the "Gold Standard" in distinguishing transplant rejection from cyclosporine nephrotoxicity. Tranplant Proc 1985; 17(Suppl 1):123-133.

16. Williams GM, White HJO, Hume DM. Factors influencing the long term functional success rate of human renal allografts. Transplantation 1967; 5:837-843.

17. Whittaker JR, Veith FJ, Soberman R, Lalezari P, Tellis I, Freed SZ, Gliedman ML. The fate of the renal transplant with delayed function. Surg Gynecol Obstet 1973; 136:919-922.

18. Cho SI, Olsson CA, Bradley JW Nasbeth DC. Regional program for kidney preservation and transplantation in New England. Am J Surg 1976; 131:428-433.

19. Opelz G, Sasaki N, Terasaki PI. Prediction of long-term kidney transplant survival rates by monitoring early graft function and clinical grades. Transplantation 1978; 25:212-215.

20. Davison JM, Uldall PR, Taylor RMR. Relation of immediate posttransplant renal function to long-term function in cadaver kidney recipients. Transplantation 1977; 23:310-315.

21. Scholz D, Horpaesy G, Mebel M. Late prognosis in acute post-transplant renal failure in 102 patients. Europ Urol 1979; 5:14-17.

22. Anderson CB, Sicard GA, Etheredge EE. Delayed primary renal function and cadaver renal allograft results. Surg Gynecol Obstet 1979;149:697-701.

23. Kjellstrand MC, Cagali RE, Simmons RL. Etiology and prognosis in acute post transplant renal failure. Am J Med 1976; 61:190-195.

24. Brophy D, Najarian JS, Kjellstrand CM. Acute tubular necrosis after renal transplantation. Transplantation 1980; 29:245-248.

25. Sanfilippo F, Vaughn WK, Spees EK, Lucas BA. The detrimental effects of delayed graft function in cadaver donor renal transplantation. Transplantation 1984; 38:643-648.

26. Sanfilippo F, Vaughn WK, LeFor WN, Spees EK. Multivariate analysis of risk factors in cadaver donor kidney transplantation. Transplantation 1986; 42:28-34.

27. McDonald JC, Vaughan W, Filo RS. Cadaver donor renal transplantation by centers of the Southeastern Organ Procurement Foundation. Ann Surg 1981; 193:1-8.

28. Besarab A, Jarrell B, Ihle BU, Colberg JE, Burke JF, Wesson L. Adverse effects of prophylactic irradiation and acute tubular necrosis on renal graft function and survival. Nephron 1982; 31:347-353.

29. Heil J, Sutherland ER, Fryd DS, Ascher NL, Simmons RL, Najarian JS. Acute tubular necrosis of cadaver renal allografts does not correlate with organ sharing or preservation time but portends a poorer prognosis for long-term graft survival. Transplant Proc 1984; 16:270-274.

30. Canafax DM, Torres A, Fryd DS, Heil JE, Strand MH, Ascher NL, Payne WD, Sutherland DER, Simmons RL, Najarian JS. The effects of delayed function on recipients of cadaver renal allografts. A study of 158 patients randomized to cyclosporine or ALG-Azathioprine. Transplantation 1986; 41:177-181.

31. Cho SI, Zalneraitis BP, Franklin C, Bradley JW. The influence of acute tubular necrosis on kidney transplant survival. Transplant Proc 1985; 17:16-17.

32. Rocher JI, Landis C, Dafoe DC, Keyserling C, Swartz RD, Campbell DA Jr. The long term deleterious effect of delayed graft function in cyclosporine-treated renal allograft recipients. Transplant Proc 1987; 19:2093-2095.

33. Kahan BD, Mickey R, Flechner SM. Multivariate analysis of risk factors impacting on immediate and eventual cadaver allograft survival in cyclosporine-treated patients. Transplantation 1987; 43:65.

34. Mendez-Picon G, Posner MP, McGeorge MB, Baquero A, Goldman MH, Monahanakumar T, Lee HM. The effect of delayed function on long term survival of renal allografts. Surg Gynecol Obstet 1985; 161:351-356.

35. Barry JM, Shively N, Hubert B, Hefty T, Norman DJ, Bennett WM. Significance of delayed graft function in cyclosporine-treated recipients of cadaver kidney transplants. 1988; 45:346-348.

36. Halloran PF, Aprile MA, Farewell V, Ludwin D, Smith EK, Tsai SY, Bear RA, Cole EH, Fenton SS, Cattran DC. Early function as the principal correlate of graft survival: A multivariate analysis of 200 cadaveric renal transplants treated with a protocol incorporating antilymphocyte globulin and cyclosporine. Transplantation 1988; 46:223-228.

37. Halloran PF, Aprile M, The Ontario Renal Transplant Research Group. Factors influencing early renal function in cadaver kidney transplants: a case-control study. Transplantation 1988; 45:122-28.

38. Halloran P, Aprile M. A randomized prospective trial of cold storage versus pulsatile perfusion for cadaver kidney preservation. Transplantation 1987; 43:827-832.

39. Canadian Multicentre Transplant Study Group. A randomized clinical trial of cyclosporine in cadaveric renal transplantation. New Eng J Med 1983; 309:809-815.

40. Pichlmayr R, Wonigeit K, Klempnauer J, Ringe B, Bunzendahl B, Kreller H, Heigel B, Siegismund S. Experience with cytlosporin/ prednisolone therapy in kidney transplantation with special reference to nephrotoxicity, ATN and kidney preservation. Neth J Med 1985; 28:268-271.

41. Salmela K, Hockerstedt EK, Pettersson E, Sarparanta T, von Willebrand E, Ahonen J. Rejection and early nonfunction in renal allografts. Transplant Proc 1986; 18:77-79.

42. Shoskes DA, Jordan ML, Cardella CJ. Prolonged simple cold storage does not adversely affect renal allograft outcome in patients receiving cyclosporine. Transplant Proc 1989; 21(1):1280-1281.

43. Lennard TWJ, Parrott NR, Wilson RG, Proud G, Farndon JR, Simpson J, Taylor RMR. Primary nonfunction after renal transplantation: A prospective multifactorial analysis of possible causes in 106 consecutive transplants. Transplant Proc 1988; 20:439-441.

44. Gorer PA. The detection of a hereditary antigenic difference in the blood of mice by means of human group A serum. J Genetics 1936; 32:17-31.

45. Snell GD. Methods for the study of histocompatibility genes. J Genetics 1948; 49:87-108.

46. Klein J. Natural history of the major histocompatibility complex. John Wiley and Sons 1986.

47. Golub ES. Immunology: A Synthesis. Sinauer Assoc, Sunderland 1987.

48. Hood L, Kronenberg M, Hunkapiller T. T cell antigen receptors and the immunoglobulin supergene family. Cell 1985; 40:225-229.

49. Roitt IM, Brostoff J, Male DK. Immunology. CV Moseby, St Louis, 1986.

50. Parnes RJ, Seidman JG. Structure of wild-type and mutant mouse β_2 genes. Cell 1982; 29:661-669.

51. Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC. Structure of the human class I histocompatiblity antigen, HLA-A2. Nature 1987; 329:506-512.

52. Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC. The foreign antigen binding site and T cell recognition regions of class I histocompatiblity antigens. Nature 1987; 329:512-518.

53. Brown JH, Jardetzky T, Saper MA, Samraoui B, Bjorkman PJ, Wiley DC. A hypothetical model of the foreign antigen binding site of Class II histocompatibility molecules. Nature 1988; 332:845-850.

54. Klein J, Juretic A, Baxevanis CN, Nagy ZA. The traditional and a new version of the mouse H-2 complex. Nature 1981; 291:455-460.

55. Lindahl KF, Wilson DB. Histocompatibility antigen activated cytotoxic T lymphocytes. I. Estimates of the frequency and specificity of precursors. J Exp Med 1977; 145:508-522.

56. Sprent J, Schaefer M. Properties of purified T cell subsets. I. *In vitro* responses to class I vs class II H-2 alloantigens. J Exp Med 1985; 162:2068-2088.

57. Doherty PC, Zinkernagel RM. A biological role for major histocompatibility antigens. Lancet 1975; 1:1406.

58. Barber B. On the interaction between MHC molecules. Immunol Today 1987; 8:9-10.

59. Babbitt BP, Allen PM, Matsueda G, Haber E, Unanue ER. Binding of immunogenic peptides to Ia histocompatibility molecules. Nature1985; 317:359-361.

60. Signaes C, Katze MG, Persson H, Philipson L. An adenovirus glycoprotein binds heavy chains of class I transplantation antigens from man and mouse. Nature 1982; 299:175-178.

61. Daar AS, Fuggle SV, Fabre JW, Ting A, Morris PJ. The detailed distribution of HLA-A,B,C antigens in normal human organs. Transplantation 1984; 38:287-292.

62. Daar AS, Fuggle SV, Fabre JW, Ting A, Morris PJ. The detailed distribution of MHC class II antigens in normal human organs. Transplantation 1984; 38:293-298.

63. Bukowski JF, Welsh RM. Interferon enhances the susceptibility of virus-infected fibroblasts to cytotoxic T cells. J Exp Med 1985; 161:257-262.

64. Flyer DC, Burakoff SJ, Faller DV. Retrovirus-induced changes in major histocompatibility complex antigen expression influence susceptibility to lysis by cytotoxic T lymphocytes. J Immunol 1985; 135:2287-2292.

65. King NJC, Mullbacher A, Blanden RV. Relationship between surface H-2 concentration, size of different target cells, and lysis by cytotoxic T cells. Cell Immunol 1986; 98:525-531.

66. Bishop GA, Waugh JA, Hall BM. Expression of HLA antigens on renal tubular cells in culture. II. Effect of increased HLA antigen expression on tubular cell stimulation of lymphocyte activation and on their vulnerability to cell-mediated lysis. Transplantation 1988; 46:303-310.

67. Bevan MJ. High determinant density may explain the phenomenon of alloreactivity. Immunol Today 1984; 5:128-130.

68. Bekkhoucha F, Naquet P, Pierres A, Marchetto S, Pierres M. Efficiency of antigen presentation to T cell clones by (B cell X B cell lymphoma) hybridomas correlates quantitatively with cell surface Ia antigen expression. Eur J Immunol 1984; 14:807-814.

69. Janeway CA, Bottomly K, Babich J, Conrad P et al. Quantitative variation in Ia antigen expression plays a central role in immune regulation. Immunol Today 1984; 5:99-105.

70. Unanue ER, Beller DI, Lu CY, Allen PM. Antigen presentation: comments on its regulation and mechanism. J Immunol 1984; 132:1-5.

71. Halloran PF, Wadgymar A, Autenried P. The regulation of expression of major histocompatibility complex products. Transplantation 1986; 41:413-420.

72. Unanue ER. Comment on the finding of Ia expression in nonlymphoid cells. Lab Invest 1986; 55:123-125.

73. Pardi R, Bender JR, Engleman EG. Lymphocyte subsets differentially induce class II human leukocyte antigens on allogeneic microvascular endothelial cells. J Immunol 1987; 139:2585-2592.

74. Sullivan KE, Calman AF, Nakanishi M, Tsang SY, Wang Y, Peterlin BM. A model for the transcriptional regulation of MHC class II genes. Immunol Today 1987; 8:289-292.

75. Kress M, Glaros D, Khoury G, Jay G. Alternative RNA splicing of the H-2K gene. Nature 1983; 306:602-604.

76. Weis JH, Seidman JG. The expression of major histocompatibility antigens under metallothionein gene promoter control. J Immunol 1985; 134:1999-2003.

77. Lindahl P, Gresser I, Leary P, Torey M. Interferon treatment of mice: enhanced expression of histocompatibility antigens on lymphoid cells. Proc Natl Acad Sci USA 1976; 73:2785.

78. Rosa F, Hatat D, Abadie A, Fellous M. Regulation of histocompatibility antigens by interferon. Ann Inst Pasteur/Immunol 1985; 136C:103-119.

79. Pober JS, Gimbrone MA, Cotran RS et al. Ia expression by vascular endothelium is inducible by activated T cells and by human gamma interferon. J Exp Med 1983; 157:1339-1353.

80. Skoskiewicz MJ, Colvin RB, Schneeberger EE, Russell PS. Widespread and selective induction of major histocompatibility complex-determined antigens *in vivo* by gamma interferon. J Exp Med 1985; 162:1645-1664.

81. Momburg F, Koch N, Möller P, Moldenhauer G, Butcher GW, Hämmerling GJ. Differential expression of Ia and Ia-associated invariant chain in mouse tissues after *in vivo* treatment with IFN-γ. J Immunol 1985; 136:940-948.

82. Gerrard TL, Dyer DR, Zoon KC, Nedden DZ, Siegel JP. Modulation of class I and class II histocompatibility antigens on human T cell lines by IFN-γ. J Immunol 1988; 140:3450-3455.

83. Sandvig S, Laskay T, Andersson J, DeLey M, Andersson U. Gamma-interferon is produced by CD3+ and CD3- lymphocytes. Immunol Rev 1987; 97:51-65.

84. Mason DW, Dallman M, Barclay AN. Graft vs Host disease induces expression of Ia antigen in rat epidermal cells and gut epithelium. Nature 1981; 293:150-151.

85. Ho M, Breinig MC, Maehara N. Cellular basis of interferon formation and hyporeactivity after exposure to bacterial lipopolysaccharide. J Infect Dis 1976; 133:30-35.

86. Ziegler KH, Staffileno LK, Wentworth P. Modulation of macrophage Ia-expression by lipopolysaccharide. I. Induction of Ia expression *in vivo*. J Immunol 1984; 133:1825-1835.

87. Lapierre LA, Fiers W, Pober JS. Three distinct classes of regulatory cytokines control endothelial cell MHC antigen expression. J Exp Med 1988; 167:794-804.

88. Mond JJ, Carman J, Sarma C, Ohara J, Finkelman FD. Interferon γ suppresses B cell stimulation factor (BSF-1) induction of class II MHC determinants on B cells. J Immunol 1986; 137:3534-3537.

89. Kahan BD. Cyclosporine: The agent and its actions. Transplant Proc 1985; 17(Suppl 1):5-18.

90. Thomson AW, Whiting PH, Simpson JG. Cyclosporine: immunology, toxicity, and pharmacology in experimental animals. Agents and Action 1984; 15:306-327.

91. Takahashi N, Toshiya H, Suzuki M. Peptidyl-prolyl *cis-trans* isomerase is the cyclosporin A-binding protein cyclophilin. Nature 1989; 337:473-475.

92. Fischer G, Wittmann-Liebold B, Lang K, Kiefhaber T, Schmid FX. Cyclophilin and peptidyl-prolyl *cis-trans* isomerase are probably identical proteins. Nature 1989; 337:476-478.

93. De Waal RMW, Bogman MJJ, Maass CN et al. Variable expression of Ia antigens on the vascular endothelium of mouse skin allografts. Nature 1983; 303:426-429.

94. Reem GH, Cook LA, Vilcek J. Gamma interferon synthesis by human thymocytes and T lymphocytes inhibited by cyclosporin A. Science 1983; 221:63-65.

95. Warren MK, Vogel SN. Opposing effects of glucocorticoids on interferon γ induced murine macrophage Fc receptor and Ia antigen expression. J Immunol 1985; 134:2462-2469.

96. Ruerstjm B, Leeuwenberg JFM, Spronken EEM, van der Linden CJ, Buurman WA. The effect of steroids on the regulation of major histocompatibility complex class II expression on nonlymphoid tissue. Transplantation 1989; 47:492-499.

97. Deeg HJ. Ultraviolet irradiation in transplantation biology. Transplantation 1988; 45:845-851.

98. Snyder DS, Beller DI, Unanue ER. Prostaglandins modulate macrophage Ia expression. Nature 1982; 299:163-165.

99. Steiniger B, Klempnauer J, Wonigeit K. Altered distribution of class I and class II MHC antigens during acute pancreas allograft rejection in the rat. Transplantation 1985; 40:234-239.

100. Milton AD, Fabre JW. Massive induction of donor-type histocompatibility complex antigens in rejecting cardiac allografts in the rat. J Exp Med 1985; 161:98-112.

101. Milton AD, Spencer SC, Fabre JW. Detailed analysis and demonstration of differences in the kinetics of induction of class I and class II major histocompatibility complex antigens in rejecting cardiac and kidney allografts in the rat. Transplantation 1986; 41:499-508.

102. Benson EM, Colvin RB, Russell PS. Induction of IA antigens in murine renal transplants. J Immunol 1985; 134:7-9.

103. Milton AD, Spencer SC, Fabre JW. The effects of cyclosporine on the induction of donor class I and class II MHC antigens in heart and kidney allografts in the rat. Transplantation 1986; 42:337-347.

104. Armstrong HE, Bolton EM, McMillan I, Spencer SC, Bradley JA. Prolonged survival of actively enhanced rat renal allografts despite accelerated cellular infiltration and rapid induction of both class I and class II MHC antigens. J Exp Med 1987; 164:891-907.

105. Wood KJ, Dallman MJ, Morris PJ. Induction of donor major histocompatibility complex antigens may not always correlate with graft rejection. Transplant Proc 1988; 20:264-266.

106. Wood KJ, Hopley A, Dallman MJ, Morris PJ. Lack of correlation between the induction of donor class I and class II major histocompatibility complex antigens and graft rejection. Transplantation 1988; 45:759-767.

107. Häyry P, von Willebrand E, Ahonen J, Eklund B. Do well-to-do and repeatedly rejecting renal allografts express the transplantation antigens similarly on their surface? Scand J Urol Nephrol 1981; Suppl 64:52-55.

108. Häyry P, Ferry B, Leszczynski D, von Willebrand E, Schellekens H. Regulation of class II MHC antigen expression and graft immunogenic potential. Transplant Proc 1986; 18:1355-1358.

109. Hall BM, Duggin GG, Philips J et al. Increased expression of HLA-DR antigens on renal tubular cells in renal transplants: Relevance to the rejection response. Lancet 1984; 2:247-251.

110. Bishop GA, Hall BM, Philips J et al. Expression of HLA-DR antigens on renal tubular cells during rejection. Transplant Proc 1985; 17:64-65.

111. Wallace AC, Keown PA, Sinclair NR. Presence of HLA-DR antigen on renal tubular cells and its relation to graft rejection. Transplant Proc 1986; 18:710-712.

112. Fuggle SV, McWhinnie DL, Chapman J, Taylor HM, Morris PJ. Sequential analysis of HLA-class II antigen expression in human renal allografts. Transplantation 1986; 42:144-150.

113. Fuggle SV, McWhinnie DL,Morris PJ. Precise specificity of induced tubular HLA-class II antigens in renal allografts. Transplantation 1987; 44:214-220.

114. Ahmed-Ansari A, Tadros TS, Knopf WD et al. Major histocompatibility complex class I and class II expression by myocytes in cardiac biopsies posttransplantation. Transplantation 1988; 45:972-978.

115. So SKS, Platt JL, Ascher NL, Snover DC. Increased expression of class I major histocompatibility complex antigens on hepatocytes in rejecting human liver allografts. Transplantation 1987; 43:79-85.

116. Sinkovics JG. Oncogenes and growth factors. CRC Crit Rev Immunol 1988; 8:217-291.

117. Bishop JM. The molecular genetics of cancer. Science 1987; 235:305-311.

118. Vennstrom B, Sheiness D, Zabielski J, Bishop JM. Isolation and characterization of c-myc, a cellular homolog of the oncogene (v-myc) of avian myelocytomatosis virus strain 29J. Virol 1982; 42:773-779.

119. Alitalo K, Ramsay G, Bishop JM et al. Identification of nuclear proteins encoded by viral and cellular myc oncogenes. Nature1983;306:272-275.

120. Persson H, Hennighausen L, Taub R, DeGRado W, Leder P. Antibodies to human c-myc oncogene product: Evidence of an evolutionarily conserved protein induced during cell proliferation. Science1984; 225:687-693.

121. Studzinski GP, Brelvi ZS, Feldman SC, Watt RA. Participation of c-myc protein in DNA synthesis of human cells. Science1986; 234:467-470.

122. Einat M, Resnitzky D, Kimchi A. Close link between reduction of c-myc expression by interferon and G0/G1 arrest. Nature1985; 313:597-600.

123. Reed JC, Alpers JD, Nowell PC, Hoover RG. Sequential expression of protooncogenes during lectin-stimulated mitogenesis of normal human lymphocytes. Proc Natl Acad Sci1986; 83:3982.

124. Reed JC, Alpers JD, Scherle PA, Hoover RG, Nowell PC, Prystowsky MB. Proto-oncogene expression in cloned T lymphocytes: mitogens and growth factors induce different patterns of expression. Oncogene1987; 1:223-228.

125. Cowley BD, Smardo FL, Grantham JJ, Calvet JP. Elevated c-myc protooncogene expression in autosomal recessive polycystic kidney disease. Proc Natl Acad Sci 1987; 84:8394-8398.

126. Makino M, Hayashi K, Sugimura T. c-myc transcript is induced in rat liver at a very early stage of regeneration or by cycloheximide treatment. Nature 1984; 310:697-698.

127. Solez K, D'Agostino RJ, Stawowy L et al. Beneficial effect of propranolol in a histologically appropriate model of postischemic acute renal failure. Am J Pathol 1977; 88:163-192.

128. Solez K, Racusen LC, Whelton A. Glomerular epithelial cell changes in early postischemic acute renal failure in rabbits and man. Am J Pathol 1981; 103:163-173.

129. Arendshorst WJ, Finn WF, Gottschalk CW. Pathogenesis of acute renal failure following temporary renal ischemia in the rat. Circ Res1975; 37:558.

130. Finn WF, Chevalier RL. Recovery from postischemic acute renal failure in the rat. Kidney Int 1979;16:113.

131. Karlberg L, Källskog Ö, Norlen BJ, Wolgast M. Nephron function in postischemic acute renal failure. Scand J Urol Nephrol 1982;16:167-172.

132. Torhurst J, Rougemont D, Brunner FP, Thiel G. Morphology of the renal medulla in ischemic acute renal failure in the rat. Nephron 1982; 31:296-300.

133. Ferwana OS, Pirie SC. Unilateral renal ischemia in the rat: effect of method of occlusion of the blood supply on function of ipsilateral and contralateral kidneys. Clin Sci 1987; 73:11-17.

134. Fox M. Progressive renal fibrosis following acute tubular necrosis: an experimental study. J Urol 1967; 97:196-202.

135. Oi VT, Jones PP, Goding JW, Herzenberg LA. Properties of monoclonal antibodies to mouse Ig allotypes, H-2, and Ia antigens. Curr Top Microbiol Immunol. 1978; 81:115-129.

136. Ledbetter JA, Herzenberg LA. Xenogenic monoclonal antibodies to mouse lymphoid differentiation antigens. Immunol Rev 1979; 47:63-90.

137. Spitalny GL, Havell EA. Monoclonal antibody to murine gamma interferon inhibits lymphokine-induced antiviral and macrophage tumoricidal activities. J Exp Med 1984; 159:1560-1565.

138. Kappler JW, Skidmore B, White J, Marrack P. Antigen-inducible, H-2 restricted, interleukin-2 producing T cell hybridomas: lack of independent antigen and H-2 recognition. J Exp Med 1981; 153:1198-1202.

139. Ozato K, Mayer NM, Sachs DH. Monoclonal antibodies to mouse major histocompatibility complex antigens. IV. A series of hybridoma clones producing anti-H-2^d antibodies and an examination of expression of H-2^d antigen on the surface of these cells. Transplantation 1982; 34:113-118.

140. Parnes JR, Velan B, Felsenfeld A et al. Mouse $ß_2$-microglobulin cDNA clones: A screening procedure for cDNA clones corresponding to rare mRNA's. Proc Natl Acad Sci USA 1981; 78:2253-2257.

141. Davis MM, Cohen DI, Nielsen E, Steinmetz M, Paul WE, Hood L. Cell-type-specific cDNA probes and the murine I region: The localization and orientation of Aα. Proc Natl Acad Sci USA 1984; 81:2194-2198.

142. Shen-Ong GLC, Keath EJ, Piccoli ST, Cole MD. Novel myc oncogene RNA from abortive immunoglobulin-gene recombination in mouse plasmacytomas. Cell 1982: 31:443-452.

143. Cleveland DW, Lopata MA, MacDonald RJ et al. Number and evolutionary conservation of α- and β- tubulin and cytoplasmic β- and γ- actin genes using specific cloned cDNA probes. Cell 1980; 20:95-105.

144. Halloran PF, Wadgymar A, Autenried P. Inhibition of MHC product induction may contribute to the immunosuppressive action of ciclosporin. Prog Allergy 1986; 38:258-268.

145. Schrader JW, Battye F, Scollay R. Expression of Thy-1 antigen is not limited to T cells in cultures of mouse hemopoietic cells. Proc Natl Acad Sci USA 1982; 79:4161-4165.

146. Mason L, Giardina SL, Hecht T, Ortaldo J, Mathieson BJ. LGL-1: A non-polymorphic antigen expressed on a major population of mouse natural killer cells. J Immunol 1988; 140:4403-4412.